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(54) Title: METHODS OF THERAPY FOR B CELL-RELATED CANCERS

(57) Abstract: Methods of treating a human subject for a cancer characterized by neoplastic B cell growth are provided. The methods comprise administering combination antibody therapy to the subject, where an effective amount of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in combination with an anti-CD20 antibody or antigen-binding fragment thereof is administered to the subject. The invention further comprises pharmaceutical compositions with combinations of these antibodies in a pharmaceutically acceptable carrier.



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## METHODS OF THERAPY FOR B CELL-RELATED CANCERS

### FIELD OF THE INVENTION

The present invention relates to methods of combination antibody therapy for B cell-related cancers, particularly cancers comprising neoplastic cells expressing the CD40 and CD20 cell surface antigens.

### BACKGROUND OF THE INVENTION

Leukemia, lymphoma, and myeloma strike over 100,000 individuals every year in the U.S. alone. A large percentage of these cases are characterized by an outgrowth of neoplastic B cells expressing the CD40 and CD20 antigens. CD40 is a 55 kDa cell-surface antigen present on the surface of both normal and neoplastic human B cells, dendritic cells, other antigen presenting cells (APCs), endothelial cells, monocytic cells, and epithelial cells. Binding of the CD40 ligand to CD40 on the B cell membrane provides a positive costimulatory signal that stimulates B cell activation and proliferation, resulting in B cell maturation into a plasma cell that secretes high levels of soluble immunoglobulin. Transformed cells from patients with low- and high-grade B cell lymphomas, B cell acute lymphoblastic leukemia, multiple myeloma, chronic lymphocytic leukemia, and Hodgkin's disease express CD40. CD40 expression is also detected in two-thirds of acute myeloblastic leukemia cases and 50% of AIDS-related lymphomas. Malignant B cells from several tumors of B-cell lineage express a high level of CD40 and appear to depend on CD40 signaling for survival and proliferation. This renders the CD40 antigen a potential target for anti-cancer therapy.

CD20 is expressed early in B cell differentiation and remains on the cell surface throughout B cell development. CD20 is involved in B cell activation, is expressed at very high levels on neoplastic B cells, and is a clinically recognized therapeutic target (see, for example, Hooijberg *et al.* (1995) *Cancer Research* 55:2627). Antibodies

targeting CD20, such as Rituxan<sup>®</sup>, have been approved by the U.S. Food and Drug Administration for the treatment of non-Hodgkin's lymphoma (see, for example, Boye *et al.* (2003) *Ann. Oncol.* 14:520). Rituxan<sup>®</sup> has been shown to be an effective treatment for low-, intermediate-, and high-grade non-Hodgkin's lymphoma (NHL) (see, for example, 5 Maloney *et al.* (1994) *Blood* 84:2457-2466); McLaughlin *et al.* (1998) *J. Clin. Oncol.* 16:2825-2833; Maloney *et al.* (1997) *Blood* 90:2188-2195; Hainsworth *et al.* (2000) *Blood* 95:3052-3056; Colombat *et al.* (2001) *Blood* 97:101-106; Coiffier *et al.* (1998) *Blood* 92:1927-1932); Foran *et al.* (2000) *J. Clin. Oncol.* 18:317-324; Anderson *et al.* (1997) *Biochem. Soc. Trans.* 25:705-708; Vose *et al.* (1999) *Ann. Oncol.* 10:58a).

10        Though the exact mechanism of action is not known, evidence indicates that the anti-lymphoma effects of Rituxan<sup>®</sup> are in part due to complement-mediated cytotoxicity (CMC), antibody-dependent cell-mediated cytotoxicity (ADCC), inhibition of cell proliferation, and finally direct induction of apoptosis. Some patients, however, become resistant to treatment with Rituxan<sup>®</sup> (Witzig *et al.* (2002) *J. Clin. Oncol.* 20:3262; Grillo- 15 Lopez *et al.* (1998) *J. Clin. Oncol.* 16:2825; Jazirehi *et al.* (2003) *Mol. Cancer Ther.* 2:1183-1193). For example, some patients lose CD20 expression on malignant B cells after anti-CD20 antibody therapy (Davis *et al.* (1999) *Clin. Cancer Res.* 5:611). Furthermore, 30% to 50% of patients with low-grade NHL exhibit no clinical response to this monoclonal antibody (Hainsworth *et al.* (2000) *Blood* 95:3052-3056; Colombat *et al.* (2001) *Blood* 97:101-106). For patients developing resistance to this monoclonal 20 antibody, or having a B cell lymphoma that is resistant to initial therapy with this antibody, alternative forms of therapeutic intervention are needed.

25        Thus, there is a need for treatment regimens for B cell-related cancers that do not create antibody resistance and which can provide effective therapy in the event antibody resistance occurs. Consequently, the discovery of a combination antibody therapy with superior anti-tumor activity compared to single-agent Rituxan<sup>®</sup> could drastically improve methods of cancer therapy for individuals with myelomas, leukemias, and lymphomas, particularly B cell lymphomas.

## BRIEF SUMMARY OF THE INVENTION

Methods of treating a subject for a cancer characterized by neoplastic B cell growth are provided. The methods comprise administering a combination of antibodies that have a therapeutic effect against neoplastic B cells expressing the CD40 and CD20 cell surface antigens. In some embodiments, a synergistic therapeutic effect occurs, making the invention especially useful for treating cancers that are refractory to antibody therapy that targets a single B cell surface antigen.

In accordance with the methods of the present invention, an individual in need thereof is administered a combination of an antagonist anti-CD40 antibody (or antigen-binding fragment thereof) and an anti-CD20 antibody (or antigen-binding fragment thereof). Suitable antagonist anti-CD40 antibodies for use in the methods of the invention include monoclonal antibodies or antigen-binding fragments thereof that are capable of specifically binding to human CD40 antigen expressed on the surface of a human cell. They are free of significant agonist activity but exhibit antagonist activity when bound to CD40 antigen on human cells, particularly when bound to CD40 antigen on neoplastic human B cells. Suitable monoclonal anti-CD40 antibodies have human constant regions; preferably they also have wholly or partially humanized framework regions; and most preferably are fully human antibodies or antigen-binding fragments thereof. Examples of such monoclonal anti-CD40 antibodies are the antibodies designated herein as CHIR-5.9 and CHIR-12.12, which can be recombinantly produced; the monoclonal antibodies produced by the hybridoma cell lines designated 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12); a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequence shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequence shown in SEQ ID NO:6 and SEQ ID NO:8; a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5; a monoclonal antibody comprising an amino acid sequence encoded by a nucleic acid

molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3; and antigen-binding fragments of these monoclonal antibodies that retain the capability of specifically binding to human CD40, and which are free of significant agonist activity but exhibit antagonist activity when bound to CD40 antigen on human cells. Examples of such monoclonal anti-CD40 antibodies also include a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 12.12 or that produced by the hybridoma cell line 5.9; a monoclonal antibody that binds to an epitope comprising residues 82-87 of the amino acid sequence shown in SEQ ID NO:10 or SEQ ID NO:12; a monoclonal antibody that competes with the monoclonal antibody CHIR-12.12 or CHIR-5.9 in a competitive binding assay; and a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 or CHIR-5.9 monoclonal antibody or any of the foregoing monoclonal antibodies, where the fragment retains the capability of specifically binding to human CD40 antigen.

Suitable anti-CD20 antibodies for practicing the invention include, but are not limited to, the chimeric monoclonal antibody IDEC-C2B8 (Rituxan<sup>®</sup> or rituximab); and anti-CD20 antibodies having the binding characteristics of IDEC-C2B8, where the anti-CD20 antibodies compete with the IDEC-C2B8 antibody in a competitive binding assay or bind to an epitope capable of binding the IDEC-C2B8 antibody. The methods of the invention are particularly effective when antagonist anti-CD40 antibodies produced by a hybridoma such as 5.9 or 12.12 are administered in combination with an anti-CD20 antibody such as IDEC-C2B8. The invention further includes pharmaceutical compositions comprising such combinations of antibodies in a pharmaceutically acceptable carrier.

The methods of the invention are useful for treating individuals with B cell lymphomas such as non-Hodgkin's lymphomas (high-grade lymphomas, intermediate-grade lymphomas, and low-grade lymphomas), Hodgkin's disease, acute lymphoblastic leukemias, myelomas, chronic lymphocytic leukemias, and myeloblastic leukemias, and are particularly useful for treatment of B cell-related cancers that are refractory to treatment with single antibody therapy that targets the CD20 cell surface antigen.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of combined administration of mAb CHIR-12.12 and mAb IDEC-C2B8 on tumor volume in a murine Rituxan<sup>®</sup>-resistant tumor model over  
5 time.

Figure 2 sets forth the amino acid sequences for the light and heavy chains of the mAb CHIR-12.12. The leader (residues 1-20 of SEQ ID NO:2), variable (residues 21-132 of SEQ ID NO:2), and constant (residues 133-239 of SEQ ID NO:2) regions of the light chain are shown in Figure 2A. The leader (residues 1-19 of SEQ ID NO:4), variable  
10 (residues 20-139 of SEQ ID NO:4), and constant (residues 140-469 of SEQ ID NO:4) regions of the heavy chain are shown in Figure 2B. The alternative constant region for the heavy chain of the mAb CHIR-12.12 shown in Figure 2B reflects a substitution of a serine residue for the alanine residue at position 153 of SEQ ID NO:4. The complete sequence for this variant of the heavy chain of the mAb CHIR-12.12 is set forth in SEQ  
15 ID NO:5.

Figure 3 shows the coding sequence for the light chain (Figure 3A; SEQ ID NO:1) and heavy chain (Figure 3B; SEQ ID NO:3) for the mAb CHIR-12.12.

Figure 4 sets forth the amino acid sequences for the light and heavy chains of mAb CHIR-5.9. The leader (residues 1-20 of SEQ ID NO:6), variable (residues 21-132  
20 of SEQ ID NO:6), and constant (residues 133-239 of SEQ ID NO:6) regions of the light chain are shown in Figure 4A. The leader (residues 1-19 of SEQ ID NO:7), variable (residues 20-144 of SEQ ID NO:7), and constant (residues 145-474 of SEQ ID NO:7) regions of the heavy chain are shown in Figure 4B. The alternative constant region for the heavy chain of the mAb CHIR-5.9 shown in Figure 4B reflects a substitution of a  
25 serine residue for the alanine residue at position 158 of SEQ ID NO:7. The complete sequence for this variant of the heavy chain of the mAb CHIR-5.9 is set forth in SEQ ID NO:8.

Figure 5 shows the coding sequence (Figure 5A; SEQ ID NO:9) for the short isoform of human CD40 (amino acid sequence shown in Figure 5B; SEQ ID NO:10), and  
30 the coding sequence (Figure 5C; SEQ ID NO:11) for the long isoform of human CD40 (amino acid sequence shown in Figure 5D; SEQ ID NO:12).

Figure 6 shows thermal melting temperature of CHIR-12.12 in different pH formulations measured by differential scanning calorimetry (DSC).

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## DETAILED DESCRIPTION OF THE INVENTION

“Tumor,” as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. “Neoplastic,” as used herein, refers to any form of dysregulated or unregulated cell growth, whether malignant or benign, resulting in abnormal tissue growth.

10

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, lymphoma and leukemia. By “B cell-related cancer” is intended any type of cancer in which the dysregulated or unregulated cell growth is associated with B cells.

15

By “refractory” in the context of a cancer is intended the particular cancer is resistant to, or non-responsive to, therapy with a particular therapeutic agent. A cancer can be refractory to therapy with a particular therapeutic agent either from the onset of treatment with the particular therapeutic agent (i.e., non-responsive to initial exposure to the therapeutic agent), or as a result of developing resistance to the therapeutic agent, either over the course of a first treatment period with the therapeutic agent or during a subsequent treatment period with the therapeutic agent.

20

“Antibodies” and “immunoglobulins” (Igs) are glycoproteins having the same structural characteristics. The terms are used synonymously. In some instances the antigen specificity of the immunoglobulin may be known.

25

The term “antibody” is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (*e.g.*, Fab, F(ab')<sub>2</sub>, Fv, single chain antibodies, diabodies, antibody chimeras, hybrid antibodies, bispecific antibodies, humanized antibodies, and the like), and recombinant peptides comprising the foregoing.

30

The terms “monoclonal antibody” and “mAb” as used herein refer to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring

mutations that may be present in minor amounts. As used herein, “anti-CD40 antibody” encompasses any antibody that specifically recognizes the CD40 cell surface antigen, including polyclonal antibodies, monoclonal antibodies, single-chain antibodies, and fragments thereof such as Fab, F(ab')<sub>2</sub>, F<sub>v</sub>, and other fragments that retain the antigen-binding function of the parent anti-CD40 antibody. Of particular interest for practicing the methods of the present invention are anti-CD40 antibodies or antigen-binding fragments thereof that have the binding properties exhibited by the CHIR-5.9 and CHIR-12.12 human anti-CD40 monoclonal antibodies described herein below.

As used herein, “anti-CD20 antibody” encompasses any antibody that specifically recognizes the CD20 cell surface antigen, including polyclonal antibodies, monoclonal antibodies, single-chain antibodies, and fragments thereof such as Fab, F(ab')<sub>2</sub>, F<sub>v</sub>, and other fragments that retain the antigen-binding function of the parent anti-CD20 antibody. Of particular interest to the methods of the present invention are anti-CD20 antibodies or antigen-binding fragments thereof that have the binding properties exhibited by the IDEC-C2B8 monoclonal antibody described herein below.

“Native antibodies” and “native immunoglobulins” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V<sub>H</sub>) followed by a number of constant domains. Each light chain has a variable domain at one end (V<sub>L</sub>) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy-chain variable domains.

The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. Variable regions confer antigen-binding specificity. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called



complementarity determining regions (CDRs) or hypervariable regions, both in the light chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called in the framework (FR) regions. The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a  $\beta$ -pleated-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the  $\beta$ -pleated-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, Kabat *et al.* (1991) *NIH Publ. No. 91-3242*, Vol. I, pages 647-669).

The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as Fc receptor binding, participation of the antibody in antibody-dependent cellular toxicity, initiation of complement dependent cytotoxicity, and mast cell degranulation.

The term "hypervariable region," when used herein, refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarily determining region" or "CDR" (i.e., residues 24-34 (L1), 50-56 (L2), and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2), and 95-102 (H3) in the heavy-chain variable domain; Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD) and/or those residues from a "hypervariable loop" (i.e., residues 26-32 (L1), 50-52 (L2), and 91-96 (L3) in the light-chain variable domain and (H1), 53-55 (H2), and 96-101 (H3) in the heavy chain variable domain; Clothia and Lesk, (1987) *J. Mol. Biol.*, 196:901-917). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues, as herein deemed.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* (1995) *Protein Eng.* 10:1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-

binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

“Fv” is the minimum antibody fragment that contains a complete antigen  
5 recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V<sub>H</sub>-V<sub>L</sub> dimer. Collectively, the six CDRs confer antigen-binding  
10 specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (C<sub>H</sub>1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain C<sub>H</sub>1 domain  
15 including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Fab' fragments are produced by reducing the F(ab')<sub>2</sub> fragment's heavy chain disulfide bridge. Other chemical couplings of antibody fragments are also known.

The “light chains” of antibodies (immunoglobulins) from any vertebrate species  
20 can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be  
25 further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Different isotypes have different effector functions.  
30 For example, IgG1 and IgG3 isotypes have ADCC (antibody dependent cell-mediated cytotoxicity) activity.

The word "label," when used herein, refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable. Radionuclides that can serve as detectable labels include, for example, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, and Pd-109. The label might also be a non-detectable entity such as a toxin.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native target disclosed herein or the transcription or translation thereof.

"Carriers," as used herein, include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN, polyethylene glycol (PEG), and Pluronics. Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive (i.e., sequential) administration in any order.

A "host cell," as used herein, refers to a microorganism or a eukaryotic cell or cell line cultured as a unicellular entity which can be, or has been, used as a recipient for a recombinant vector or other transfer polynucleotides, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

“Human effector cells” are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out antigen-dependent cell-mediated cytotoxicity (ADCC) effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, macrophages, eosinophils, and neutrophils; with PBMCs and NK cells being preferred. Antibodies that have ADCC activity are typically of the IgG1 or IgG3 isotype. Note that in addition to isolating IgG1 and IgG3 antibodies, such ADCC-mediating antibodies can be made by engineering a variable region from a non-ADCC antibody or variable region fragment onto an IgG1 or IgG3 isotype constant region.

The terms “Fc receptor” or “FcR” are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. *See*, Daeron (1997) *Annu. Rev. Immunol.* 15:203-234. FcRs are reviewed in Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-92; Capel *et al.* (1994) *Immunomethods* 4:25-34; and de Haas *et al.* (1995) *J. Lab. Clin. Med.* 126:330-41. Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.* (1976) *J. Immunol.* 117:587 and Kim *et al.* (1994). *J. Immunol.* 24:249).

The term “synergy” is used to describe a combined effect of two or more active agents that is greater than the sum of the individual effects of each respective active agent. Thus, where the combined effect of two or more agents results in “synergistic inhibition” of an activity or process, for example, tumor growth, it is intended that the inhibition of the activity or process is greater than the sum of the inhibitory effects of

each respective active agent. The term “synergistic therapeutic effect” refers to a therapeutic effect observed with a combination of two or more therapies wherein the therapeutic effect (as measured by any of a number of parameters) is greater than the sum of the individual therapeutic effects observed with the respective individual therapies.

5           The terms “therapeutically effective dose,” “therapeutically effective amount,” or “effective amount” are intended to mean an amount of the antagonist anti-CD40 antibody (or antigen-binding fragment thereof) that, when administered in combination with an amount of the anti-CD20 antibody (or antigen-binding fragment thereof), brings about a positive therapeutic response with respect to treatment of a subject for a cancer  
10       comprising neoplastic B cells.

#### Combination Therapy with Anti-CD40 and Anti-CD20 Antibodies

The present invention is directed to methods for treating a subject having a cancer characterized by neoplastic B cell growth. Such neoplastic B cells include, but are not  
15       limited to, neoplastic B cells derived from lymphomas including low-, intermediate-, and high-grade B cell lymphomas, immunoblastic lymphomas, non-Hodgkin’s lymphomas, Hodgkin’s disease, Epstein-Barr Virus (EBV) induced lymphomas, and AIDS-related lymphomas, as well as B cell acute lymphoblastic leukemias, myelomas, chronic lymphocytic leukemias, acute myeloblastic leukemias, and the like.

20           The methods of the invention encompass combination antibody therapy with an antagonist anti-CD40 antibody, or antigen-binding fragment thereof, and an anti-CD20 antibody, or antigen-binding fragment thereof. The methods of the invention are especially useful for the treatment of cancers comprising neoplastic B cells expressing both the CD40 and CD20 cell surface antigens, such as B cell lymphomas. Examples of  
25       lymphomas that may express the CD40 and CD20 antigen(s) include, but are not limited to, B cell acute lymphoblastic leukemia, Hodgkin’s disease, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, immunoblastic lymphoma, AIDS-related lymphoma, and  
30       the like.

Thus, the methods of the invention find use in the treatment of non-Hodgkin's lymphomas related to abnormal, uncontrollable B cell proliferation or accumulation. For purposes of the present invention, such lymphomas will be referred to according to the *Working Formulation* classification scheme, that is those B cell lymphomas categorized as low grade, intermediate grade, and high grade (see "The Non-Hodgkin's Lymphoma Pathologic Classification Project," *Cancer* 49(1982):2112-2135). Thus, low-grade B cell lymphomas include small lymphocytic, follicular small-cleaved cell, and follicular mixed small-cleaved and large cell lymphomas; intermediate-grade lymphomas include follicular large cell, diffuse small cleaved cell, diffuse mixed small and large cell, and diffuse large cell lymphomas; and high-grade lymphomas include large cell immunoblastic, lymphoblastic, and small non-cleaved cell lymphomas of the Burkitt's and non-Burkitt's type.

It is recognized that the methods of the invention are useful in the therapeutic treatment of B cell lymphomas that are classified according to the Revised European and American Lymphoma Classification (REAL) system. Such B cell lymphomas include, but are not limited to, lymphomas classified as precursor B cell neoplasms, such as B lymphoblastic leukemia/lymphoma; peripheral B cell neoplasms, including B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, lymphoplasmacytoid lymphoma/immunocytoma, mantle cell lymphoma (MCL), follicle center lymphoma (follicular) (including diffuse small cell, diffuse mixed small and large cell, and diffuse large cell lymphomas), marginal zone B cell lymphoma (including extranodal, nodal, and splenic types), hairy cell leukemia, plasmacytoma/ myeloma, diffuse large cell B cell lymphoma of the subtype primary mediastinal (thymic), Burkitt's lymphoma, and Burkitt's like high-grade B cell lymphoma; acute leukemias; acute lymphocytic leukemias; myeloblastic leukemias; acute myelocytic leukemias; promyelocytic leukemia; myelomonocytic leukemia; monocytic leukemia; erythroleukemia; granulocytic leukemia (chronic myelocytic leukemia); chronic lymphocytic leukemia; polycythemia vera; multiple myeloma; Waldenstrom's macroglobulinemia; heavy chain disease; and unclassifiable low-grade or high-grade B cell lymphomas.

In particular, the methods of the invention are useful for treating B cell lymphomas, including those listed above, that are refractory to (i.e., resistant to, or have

become resistant to) first-line oncotherapeutic treatments. The term “oncotherapeutic” is intended to mean a treatment for cancer such as chemotherapy, surgery, radiation therapy, single anti-cancer antibody therapy, and combinations thereof.

“Treatment” is herein defined as the application or administration of an antagonist anti-CD40 antibody or antigen-binding fragment thereof to a subject, or application or administration of an antagonist anti-CD40 antibody or fragment thereof to an isolated tissue or cell line from a subject, in combination with the application or administration of an anti-CD20 antibody or antigen-binding fragment thereof to the subject, or to an isolated tissue or cell line from the subject, where the subject has a disease, a symptom of a disease, or a predisposition toward a disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease. By “treatment” is also intended the combination of these antibodies or antigen-binding fragments thereof can be applied or administered to the subject, or to the isolated tissue or cell line from the subject, as part of a single pharmaceutical composition, or alternatively as part of individual pharmaceutical compositions, each comprising either the anti-CD40 antibody (or antigen binding fragment thereof) or anti-CD20 antibody (or antigen-binding fragment thereof), where the subject has a disease, a symptom of a disease, or a predisposition toward a disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease.

Anti-CD40 antibodies suitable for use in the methods of the invention specifically bind a human CD40 antigen expressed on the surface of a human cell and are free of significant agonist activity but exhibit antagonist activity when bound to the CD40 antigen on a human CD40-expressing cell, including normal and neoplastic (whether malignant or benign) human B cells. In some embodiments, their binding to CD40 displayed on the surface of human cells results in inhibition of proliferation and differentiation of these human cells. Thus, the antagonist anti-CD40 antibodies suitable for use in the methods of the invention include those monoclonal antibodies that can exhibit antagonist activity toward normal and neoplastic human cells expressing the cell-surface CD40 antigen. These anti-CD40 antibodies and antigen-binding fragments

thereof are referred to herein as “antagonist anti-CD40 antibodies.” Such antibodies include, but are not limited to, the fully human monoclonal antibodies CHIR-5.9 and CHIR-12.12 described below and monoclonal antibodies having the binding characteristics of monoclonal antibodies CHIR-5.9 and CHIR-12.12. These monoclonal antibodies, which can be recombinantly produced, are described below and disclosed in copending provisional applications entitled “*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*,” filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety.

In addition to the monoclonal antibodies CHIR-5.9 and CHIR-12.12, other anti-CD40 antibodies that would be useful in practicing the methods of the invention described herein include, but are not limited to: (1) the monoclonal antibodies produced by the hybridoma cell lines designated 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12), deposited with the ATCC as Patent Deposit No. PTA-5542 and Patent Deposit No. PTA-5543, respectively; (2) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5; (3) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequences shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequences shown in SEQ ID NO:6 and SEQ ID NO:8; (4) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in SEQ ID NO:1, the nucleotide sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3; (5) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody



produced by the hybridoma cell line 5.9 or the hybridoma cell line 12.12; (6) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the amino acid sequence shown in SEQ ID NO:10 or SEQ ID NO:12; (7) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay; and (8) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 or CHIR-5.9 monoclonal antibody or the foregoing monoclonal antibodies in preceding items (1)-(7), where the fragment retains the capability of specifically binding to the human CD40 antigen. Those skilled in the art recognize that the antibodies and antigen-binding fragments of these antibodies suitable for use in the methods disclosed herein include antibodies and antigen-binding fragments thereof that are produced recombinantly using methods well known in the art and described herein below, and include, for example, monoclonal antibodies CHIR-5.9 and CHIR-12.12 that have been recombinantly produced.

Anti-CD20 antibodies suitable for use in the methods of the invention specifically bind a human CD20 antigen expressed on the surface of a human cell. The anti-CD20 antibodies useful in the practice of the invention can have one or many mechanisms of action. Although the methods of the invention are not bound by any particular mechanism of action, anti-CD20 antibodies have been shown to induce at least antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), down-regulation of proliferation, and apoptosis in target cells. Such antibodies include, but are not limited to, the antibody IDEC-C2B8 (Biogen Idec Pharmaceuticals Corp., Cambridge, Massachusetts; commercially available under the tradename Rituxan<sup>®</sup>, also referred to as rituximab), which is a chimeric anti-CD20 monoclonal antibody containing human IgG1 and kappa constant regions with murine variable regions isolated from a murine anti-CD20 monoclonal antibody, IDEC-2B8 (Reff *et al.* (1994) *Blood* 83:435-445; see also U.S. Patent No. 5,736,137); radiolabeled anti-CD20 antibody Zevalin<sup>®</sup> (Ibritumomab tiuxetan), manufactured by Biogen Idec Pharmaceuticals Corp. (Cambridge, Massachusetts); Bexxar<sup>®</sup> (Tositumomab, which is the murine version of rituximab, combined with Iodine (I-131)-labeled Tositumomab), manufactured by Corixa Corp. (Seattle Washington); the fully human antibody HuMax-CD20; R-1594; IMMU-106; TRU-015; AME-133; and monoclonal antibodies having the binding characteristics

of IDEC-C2B8, that is the binding specificity of IDEC-C2B8 and capability of inducing one or more of the following activities when bound to CD20 antigen on CD20-expressing B cells: (1) antibody-dependent cell-mediated cytotoxicity (ADCC); (2) complement-dependent cytotoxicity (CDC), (3) down-regulation of B cell proliferation; and (4) apoptosis in target cells. *In vitro* and *in vivo* assays for measuring the ability of anti-CD20 antibodies to induce these activities are well known in the art. See, for example, the assays disclosed in U.S. Patent No. 5,736,137, herein incorporated by reference in its entirety. Other antibodies useful in practicing the methods of the invention are murine and human anti-CD20 antibodies conjugated to radiolabels such as In-111 and Y-90 and other therapeutic agents such as toxins.

In addition to using the antagonist anti-CD40 antibodies and the anti-CD20 antibodies mentioned above, and described more fully herein below, the methods of the present invention can be practiced using antibodies that have the binding characteristics of monoclonal antibodies CHIR-5.9, CHIR-12.12, or IDEC-C2B8 and competitively interfere with binding of these antibodies to their respective antigens or bind the same epitopes. One of skill in the art could determine whether an antibody competitively interferes with CHIR-5.9, CHIR-12.12, or IDEC-C2B8 binding using standard methods.

Combination therapy with anti-CD20 antibodies (or antigen-binding fragments thereof) and antagonist anti-CD40 antibodies (or antigen-binding fragments thereof) provides a therapeutic benefit that is greater than that provided by the use of either of these anti-cancer agents alone. In addition, these two types of antibodies can be used in combination to treat tumors that are refractory to treatment with single antibody therapy, particularly anti-CD20 antibody therapy, either as a result of initial resistance to the single antibody therapy or as a result of resistance that develops during one or more time courses of therapy with the single antibody. In yet other embodiments, combination therapy with these two antibodies has a synergistic therapeutic effect against tumors that are refractory or non-refractory (i.e., responsive) to single antibody therapy. In some embodiments, the methods of the invention comprise combination therapy with the anti-CD20 monoclonal antibody IDEC-C2B8 and the anti-CD40 monoclonal antibody CHIR-12.12. In other embodiments, the methods of the invention comprise combination therapy with the anti-CD20 monoclonal antibody IDEC-C2B8 and the anti-CD40

monoclonal antibody CHIR-5.9. In yet other embodiments, the methods of the invention comprise combination therapy with an antigen-binding fragment of the anti-CD20 monoclonal antibody IDEC-C2B8 and an antigen-binding fragment of the anti-CD40 monoclonal antibody CHIR-12.12. or CHIR-5.9. In alternative embodiments, the methods of the invention comprise combination therapy with the anti-CD20 monoclonal antibody IDEC-C2B8 and an antigen-binding fragment of the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9. In other embodiments, the methods of the invention comprise combination therapy with an antigen-binding fragment of the anti-CD20 monoclonal antibody IDEC-C2B8 and the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9. The combination therapy described herein may comprise other variations, as long as both the CD20 and CD40 antigen are targeted in the treatment process.

Multiple parameters can be indicative of treatment efficacy. These include, but are not limited to, a reduction in the size of the tumor mass; a reduction in metastatic invasiveness of the tumor; a reduction in the rate of tumor growth; a decrease in severity or incidence of tumor-related sequelae such as cachexia and ascites production; a decrease and/or prevention of tumor-related complications such as pathologic bone fractures, autoimmune hemolytic anemia, prolymphocytic transformation, Richter's syndrome, and the like; sensitization of the tumor to chemotherapy and other treatments; an increased patient survival rate; an increase in observed clinical correlates of improved prognosis such as increased tumor infiltrating lymphocytes and decreased tumor vascularization; and the like. Thus, in some embodiments, administration of the combination of these two types of antibodies will result in an improvement of one or more of these parameters in a patient (i.e., subject) undergoing treatment. In other embodiments, the improvements in the patient will be synergistic with regard to some parameters, but additive with regard to others.

By "positive therapeutic response" with respect to cancer treatment is intended an improvement in the disease in association with the anti-tumor activity of these antibodies or fragments thereof, and/or an improvement in the symptoms associated with the disease. That is, an anti-proliferative effect, the prevention of further tumor outgrowths, a reduction in tumor size, a reduction in the number of cancer cells, and/or a decrease in one or more symptoms mediated by neoplastic B cells can be observed. Thus, for

example, an improvement in the disease may be characterized as a complete response. By “complete response” is intended an absence of clinically detectable disease with normalization of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF). Such a response must persist for at least one month following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By “partial response” is intended at least about a 50% decrease in all measurable tumor burden (i.e., the number of tumor cells present in the subject) in the absence of new lesions and persisting for at least one month. Such a response is applicable to measurable tumors only.

Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, flow cytometry or fluorescence-activated cell sorter (FACS) analysis, bioluminescent imaging, for example, luciferase imaging, bone scan imaging, and tumor biopsy sampling including bone marrow aspiration (BMA). In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease. Thus, for B cell tumors, the subject may experience a decrease in the so-called B symptoms, i.e., night sweats, fever, weight loss, and/or urticaria.

By “therapeutically effective dose,” “therapeutically effective amount,” or “effective amount” is intended an amount of the antagonist anti-CD40 antibody (or antigen-binding fragment thereof) that, when administered in combination with an amount of the anti-CD20 antibody (or antigen-binding fragment thereof), brings about a positive therapeutic response with respect to treatment of a subject for a cancer comprising neoplastic B cells. In some embodiments of the invention, a therapeutically effective dose of either the anti-CD20 antibody (or antigen-binding fragment thereof) or antagonist anti-CD40 antibody (or antigen-binding fragment thereof) is in the range from about 0.01 mg/kg to about 40 mg/kg, from about 0.01 mg/kg to about 30 mg/kg, from about 0.1 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 25 mg/kg, from about 3 mg/kg to about 20 mg/kg, from about 5 mg/kg to about 15 mg/kg, or from about 7 mg/kg to about

12 mg/kg. It is recognized that the method of treatment may comprise a single administration of a therapeutically effective dose of the antibody combination useful in the practice of the invention or multiple administrations of a therapeutically effective dose of the antibody combination.

5           One method of predicting clinical efficacy is to measure the effects of combination therapy with these antibodies in a suitable model; for example, the use of the combination of an anti-CD20 antibody and an antagonist anti-CD40 antibody in murine cancer models. These models include the nude mouse xenograft tumor models such as those using the human Burkitt's lymphoma cell lines known as Namalwa and Daudi. In  
10           some embodiments, anti-tumor activity is assayed in a staged nude mouse xenograft tumor model using the Daudi human lymphoma cell line as described in U.S. Patent Application Serial No. 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)). A staged nude mouse xenograft tumor model cell line is generally more effective at distinguishing the therapeutic efficacy of a given antibody than is an  
15           unstaged model, as in the staged model antibody dosing is initiated only after the tumor has reached a measurable size. In the unstaged model, antibody dosing is initiated generally within about 1 day of tumor inoculation and before a palpable tumor is present. The ability of an antibody to exhibit increased anti-tumor activity in a staged model is a strong indication that the antibody will be therapeutically effective.

20           The methods of the invention comprise using combination therapy. The term "combination" is used in its broadest sense and means that a subject is treated with at least two therapeutic regimens. Thus, "combination antibody therapy" is intended to mean a subject is treated with at least two antibody regimens, more particularly, with at least one anti-CD20 antibody (or antigen-binding fragment thereof) in combination with  
25           at least one anti-CD40 antibody (or antigen-binding fragment thereof), but the timing of administration of the different antibody regimens can be varied so long as the beneficial effects of the combination of these antibodies is achieved. Treatment with an anti-CD20 antibody (or antigen-binding fragment thereof) in combination with an antagonist anti-CD40 antibody (or antigen-binding fragment thereof) can be simultaneous (concurrent),  
30           consecutive (sequential), or a combination thereof. Therefore, a subject undergoing combination antibody therapy can receive both antibodies at the same time (i.e.,

simultaneously) or at different times (i.e., sequentially, in either order, on the same day, or on different days), so long as the therapeutic effect of the combination of both substances is caused in the subject undergoing therapy. In some embodiments, the combination of antibodies will be given simultaneously for one dosing, but other dosings will include sequential administration, in either order, on the same day, or on different days. Sequential administration may be performed regardless of whether the subject responds to the first monoclonal antibody administration. Where the two antibodies are administered simultaneously, they can be administered as separate pharmaceutical compositions, each comprising either the anti-CD20 antibody (or antigen-binding fragment thereof) or the antagonist anti-CD40 antibody (or antigen-binding fragment thereof), or can be administered as a single pharmaceutical composition comprising both of these anti-cancer agents.

Moreover, the treatment can be accomplished with varying doses as well as dosage regimens. In some embodiments, the dose of one monoclonal antibody will differ from the dose administered for the other monoclonal antibody, as long as the combination of these doses is effective at treating any one or more of a number of therapeutic parameters. These treatment regimens are based on doses and dosing schedules that maximize therapeutic effects, such as those described above. Those skilled in the art recognize that a dose of any one monoclonal antibody may not be therapeutically effective when administered individually, but will be therapeutically effective when administered in combination with the other antibody. See, for example, Figure 1 in which anti-CD20 antibody administered alone was therapeutically ineffective, while the antagonist anti-CD40 antibody administered alone significantly inhibited the growth of the rituximab-resistant human lymphoma xenograft. When these two antibodies were administered in combination, synergistic anti-tumor activity was observed. Thus, in some embodiments, the therapeutically effective dose of a combination of anti-CD20 antibody and antagonistic anti-CD40 antibody may comprise doses of individual active agents that, when administered alone, would not be therapeutically effective or would be less therapeutically effective than when administered in combination with each other.

In some embodiments, the antibodies can be administered in equivalent amounts. Thus, where an equivalent dosing regimen is contemplated, the antagonist anti-CD40

antibody, for example, the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9, is dosed at about 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 5 mg/kg, 7 mg/kg, or 10 mg/kg, and the anti-CD20 antibody, for example, IDEC-C2B8 (Rituxan<sup>®</sup>) is also dosed at the equivalent dose of about 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 5 mg/kg, 7 mg/kg, and 10 mg/kg, respectively. In other embodiments, these antibodies can be administered in non-equivalent amounts.

Those skilled in the art recognize that the methods of combination antibody therapy disclosed herein may be used before, after, or concurrently with other forms of oncotherapy. Such oncotherapy can include chemotherapy regimens such as treatment with CVP (cyclophosphamide, vincristine and prednisone), CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), ICE (ifosfamide, carboplatin, and etoposide), Mitozantrone, Cytarabine, DVP (daunorubicin, prednisone, and vincristine), ATRA (all-trans-retinoic acid), Idarubicin, hoelzer chemotherapy regime, La La chemotherapy regime, ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine), CEOP (cyclophosphamide, epirubicin, vincristine, and prednisone), CEOP-BE (cyclophosphamide, epirubicin, vincristine, prednisone, bleomycin, and etoposide), 2-CdA (2-chlorodeoxyadenosine (2-CDA), FLAG & IDA (fludarabine, cytarabine, and idarubicin; with or without subsequent G-CSF treatment), VAD (vincristine, doxorubicin, and dexamethasone), M & P (melphalan and prednisone), C-Weekly (cyclophosphamide and prednisone), ABCM (adriamycin (doxorubicin), BCNU, cyclophosphamide, and melphalan), MOPP (nitrogen mustard, oncovin, procarbazine, and prednisone), and DHAP (dexamethasone, high-dose ara-C, and platinol). Alternatively, such oncotherapies can include radiation treatment, including myeloablative therapies. Thus, the methods of the invention find use as a concurrent treatment to kill residual tumor cells, either *in vivo* or *ex vivo*, after such oncotherapies.

#### Antagonist Anti-CD40 Antibodies

The monoclonal antibodies CHIR-5.9 and CHIR-12.12 represent suitable antagonist anti-CD40 antibodies for use in the methods of the present invention. The

CHIR-5.9 and 12.2 antibodies are fully human anti-CD40 monoclonal antibodies of the IgG<sub>1</sub> isotype produced from the hybridoma cell lines 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12). These cell lines were created using splenocytes from immunized xenotypic mice containing the human IgG<sub>1</sub> heavy chain locus and the human  $\kappa$  chain locus (Abgenix). The spleen cells were fused with the mouse myeloma SP2/0 cells (Sierra BioSource). The resulting hybridomas were sub-cloned several times to create the stable monoclonal cell lines 5.9 and 12.12. Other antibodies of the invention may be prepared similarly using mice transgenic for human immunoglobulin loci or by other methods known in the art and/or described herein.

The nucleotide and amino acid sequences of the variable regions of the CHIR-12.12 antibody, and the amino acid sequences of the variable regions of the CHIR-5.9 antibody, are disclosed in copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety. The amino acid sequences for the leader, variable, and constant regions for the light chain and heavy chain for mAb CHIR-12.12 are set forth herein in Figures 2A and 2B, respectively. See also SEQ ID NO:2 (complete sequence for the light chain of mAb CHIR-12.12), SEQ ID NO:4 (complete sequence for the heavy chain for mAb CHIR-12.12), and SEQ ID NO:5 (complete sequence for a variant of the heavy chain for mAb CHIR-12.12 set forth in SEQ ID NO:4, where the variant comprises a serine substitution for the alanine residue at position 153 of SEQ ID NO:4). The nucleotide sequences encoding the light chain and heavy chain for mAb CHIR-12.12 are set forth herein in Figures 3A and 3B, respectively. See also SEQ ID NO:1 (coding sequence for the light chain for mAb CHIR-12.12), and SEQ ID NO:3 (coding sequence for the heavy chain for mAb CHIR-12.12). The amino acid sequences for the leader, variable, and constant regions for the light chain and heavy chain of the CHIR-5.9 mAb are set forth herein in Figures 4A and 4B, respectively. See also SEQ ID



NO:6 (complete sequence for the light chain of mAb CHIR-5.9), SEQ ID NO:7 (complete sequence for the heavy chain of mAb CHIR-5.9), and SEQ ID NO:8 (complete sequence for a variant of the heavy chain of mAb CHIR-5.9 set forth in SEQ ID NO:7, where the variant comprises a serine substitution for the alanine residue at position 158 of SEQ ID NO:7). Further, hybridomas expressing CHIR-5.9 and CHIR-12.12 antibodies have been deposited with the ATCC with a patent deposit designation of PTA-5542 and PTA-5543, respectively.

In addition to antagonist activity, anti-CD40 antibodies can have another mechanism of action against a tumor cell. For example, native CHIR-5.9 and CHIR-12.12 antibodies have ADCC activity. Alternatively, the variable regions of the CHIR-5.9 and CHIR-12.12 antibodies can be expressed on another antibody isotype that has ADCC activity. It is also possible to conjugate native forms, recombinant forms, or antigen-binding fragments of CHIR-5.9 or CHIR-12.12 to a cytotoxin, therapeutic agent, or radioisotope.

The CHIR-5.9 and CHIR-12.12 monoclonal antibodies bind soluble CD40 in ELISA-type assays, prevent the binding of CD40-ligand to cell-surface CD40, and displace the pre-bound CD40-ligand, as determined by flow cytometric assays. Antibodies CHIR-5.9 and CHIR-12.12 compete with each other for binding to CD40 but not with 15B8, the anti-CD40 monoclonal antibody described in U.S. Provisional Application Serial No. 60/237,556, titled "*Human Anti-CD40 Antibodies*," filed October 2, 2000, and PCT International Application No. PCT/US01/30857, also titled "*Human Anti-CD40 Antibodies*," filed October 2, 2001 (Attorney Docket No. PP16092.003), both of which are herein incorporated by reference in their entirety. When tested *in vitro* for effects on proliferation of B cells from normal human subjects, CHIR-5.9 and CHIR-12.12 act as antagonistic anti-CD40 antibodies. Furthermore, CHIR-5.9 and CHIR-12.12 do not induce strong proliferation of human lymphocytes from normal subjects. These antibodies are able to kill CD40-expressing target cells by antibody dependent cellular cytotoxicity (ADCC). The binding affinity of CHIR-5.9 for human CD40 is  $1.2 \times 10^{-8}$  M and the binding affinity of CHIR-12.12 is  $5 \times 10^{-10}$  M, as determined by the Biacore™ assay.

Suitable antagonist anti-CD40 antibodies for use in the methods of the present invention exhibit a strong single-site binding affinity for the CD40 cell-surface antigen. The monoclonal antibodies of the invention exhibit a dissociation equilibrium constant ( $K_D$ ) for CD40 of at least  $10^{-5}$  M, at least  $3 \times 10^{-5}$  M, preferably at least  $10^{-6}$  M to  $10^{-7}$  M, more preferably at least  $10^{-8}$  M to about  $10^{-12}$  M, measured using a standard assay such as Biacore™. Biacore analysis is known in the art and details are provided in the “BIAapplications handbook.” Methods described in WO 01/27160 can be used to modulate the binding affinity.

By “CD40 antigen,” “CD40 cell surface antigen,” “CD40 receptor,” or “CD40” is intended a transmembrane glycoprotein that belongs to the tumor necrosis factor (TNF) receptor family (see, for example, U.S. Patent Nos. 5,674,492 and 4,708,871; Stamenkovic *et al.* (1989) *EMBO* 8:1403; Clark (1990) *Tissue Antigens* 36:33; Barclay *et al.* (1997) *The Leucocyte Antigen Facts Book* (2d ed.; Academic Press, San Diego)). Two isoforms of human CD40, encoded by alternatively spliced transcript variants of this gene, have been identified. The first isoform (also known as the “long isoforms” or “isoform 1”) is expressed as a 277-amino-acid precursor polypeptide (SEQ ID NO:12 (first reported as GenBank Accession No. CAA43045, and identified as isoform 1 in GenBank Accession No. NP\_001241), encoded by SEQ ID NO:11 (see GenBank Accession Nos. X60592 and NM\_001250)), which has a signal sequence represented by the first 19 residues. The second isoform (also known as the “short isoforms” or “isoform 2”) is expressed as a 203-amino-acid precursor polypeptide (SEQ ID NO:10 (GenBank Accession No. NP\_690593), encoded by SEQ ID NO:9 (GenBank Accession No. NM\_152854)), which also has a signal sequence represented by the first 19 residues. The precursor polypeptides of these two isoforms of human CD40 share in common their first 165 residues (i.e., residues 1-165 of SEQ ID NO:10 and SEQ ID NO:12). The precursor polypeptide of the short isoform (shown in SEQ ID NO:10) is encoded by a transcript variant (SEQ ID NO:9) that lacks a coding segment, which leads to a translation frame shift; the resulting CD40 isoform contains a shorter and distinct C-terminus (residues 166-203 of SEQ ID NO:10) from that contained in the long isoform of CD40 (C-terminus shown in residues 166-277 of SEQ ID NO:12). For purposes of the present invention, the term “CD40 antigen,” “CD40 cell surface antigen,” “CD40

receptor,” or “CD40” encompasses both the short and long isoforms of CD40. The anti-CD40 antibodies of the present invention bind to an epitope of human CD40 that resides at the same location within either the short isoform or long isoform of this cell surface antigen as noted herein below.

5           The CD40 antigen is displayed on the surface of a variety of cell types, as described elsewhere herein. By “displayed on the surface” and “expressed on the surface” is intended that all or a portion of the CD40 antigen is exposed to the exterior of the cell. The displayed or expressed CD40 antigen may be fully or partially glycosylated.

10           By “agonist activity” is intended that the substance functions as an agonist. An agonist combines with a cognate receptor on a cell and initiates a reaction or activity that is similar to or the same as that initiated by the receptor’s natural ligand; e.g., it transduces a signal to the cell. An agonist of CD40 induces any or all of, but not limited to, the following responses: B cell proliferation and differentiation, antibody production, intercellular adhesion, B cell memory generation, isotype switching, up-regulation of  
15           cell-surface expression of MHC Class II and CD80/86, and secretion of pro-inflammatory cytokines such as IL-8, IL-12, and TNF. By “antagonist activity” is intended that the substance functions as an antagonist. An antagonist of CD40 prevents or reduces induction of any of the responses induced by binding of the CD40 receptor to an agonist ligand, particularly CD40L. The antagonist may reduce induction of any one or more of  
20           the responses to agonist binding by 5%, 10%, 15%, 20%, 25%, 30%, 35%, preferably 40%, 45%, 50%, 55%, 60%, more preferably 70%, 80%, 85%, and most preferably 90%, 95%, 99%, or 100%. Methods for measuring anti-CD40 antibody and CD40-ligand binding specificity and antagonist activity are known to one of skill in the art and include, but are not limited to, standard competitive binding assays, assays for monitoring  
25           immunoglobulin secretion by B cells, B cell proliferation assays, Banchereau-Like-B cell proliferation assays, T cell helper assays for antibody production, co-stimulation of B cell proliferation assays, and assays for up-regulation of B cell activation markers. See, for example, such assays disclosed in WO 00/75348 and U.S. Patent No. 6,087,329, herein incorporated by reference.

30           By “significant” agonist activity is intended an agonist activity of at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the

agonist activity induced by a neutral substance or negative control as measured in an assay of a B cell response. Preferably, "significant" agonist activity is an agonist activity that is at least 2-fold greater or at least 3-fold greater than the agonist activity induced by a neutral substance or negative control as measured in an assay of a B cell response.

5 Thus, for example, where the B cell response of interest is B cell proliferation, "significant" agonist activity would be induction of a level of B cell proliferation that is at least 2-fold greater or at least 3-fold greater than the level of B cell proliferation induced by a neutral substance or negative control. In one embodiment, a non-specific immunoglobulin, for example IgG1, that does not bind to CD40 serves as the negative  
10 control. A substance "free of significant agonist activity" would exhibit an agonist activity of not more than about 25% greater than the agonist activity induced by a neutral substance or negative control, preferably not more than about 20% greater, 15% greater, 10% greater, 5% greater, 1% greater, 0.5% greater, or even not more than about 0.1% greater than the agonist activity induced by a neutral substance or negative control as  
15 measured in an assay of a B cell response. The antagonist anti-CD40 antibodies useful in the methods of the present invention are free of significant agonist activity as noted above when bound to a CD40 antigen on a human cell. In one embodiment of the invention, the antagonist anti-CD40 antibody is free of significant agonist activity in one B cell response. In another embodiment of the invention, the antagonist anti-CD40 antibody is  
20 free of significant agonist activity in assays of more than one B cell response (e.g., proliferation and differentiation, or proliferation, differentiation, and antibody production).

Monoclonal antibodies to CD40 are known in the art. See, for example, the sections dedicated to B-cell antigen in McMichael, ed. (1987; 1989) *Leukocyte Typing III and IV* (Oxford University Press, New York); U.S. Patent Nos. 5,674,492; 5,874,082; 5,677,165; 6,056,959; WO 00/63395; International Publication Nos. WO 02/28905 and WO 02/28904; Gordon *et al.* (1988) *J. Immunol.* 140:1425; Valle *et al.* (1989) *Eur. J. Immunol.* 19:1463; Clark *et al.* (1986) *PNAS* 83:4494; Paulie *et al.* (1989) *J. Immunol.* 142:590; Gordon *et al.* (1987) *Eur. J. Immunol.* 17:1535; Jabara *et al.* (1990) *J. Exp. Med.* 172:1861; Zhang *et al.* (1991) *J. Immunol.* 146:1836; Gascan *et al.* (1991) *J. Immunol.* 147:8; Banchereau *et al.* (1991) *Clin. Immunol. Spectrum* 3:8; and Banchereau

*et al.* (1991) *Science* 251:70; all of which are herein incorporated by reference. Of particular interest to the present invention are the antagonist anti-CD40 antibodies disclosed herein that share the binding characteristics of the monoclonal antibodies CHIR-5.9 and CHIR-12.12 described above. Such antibodies include, but are not limited to the following: (1) the monoclonal antibodies produced by the hybridoma cell lines designated 131.2F8.5.9 (referred to herein as the cell line 5.9) and 153.8E2.D10.D6.12.12 (referred to herein as the cell line 12.12), deposited with the ATCC as Patent Deposit No. PTA-5542 and Patent Deposit No. PTA-5543, respectively; (2) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5; (3) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequences shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequences shown in SEQ ID NO:6 and SEQ ID NO:8; (4) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in SEQ ID NO:1, the nucleotide sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3; (5) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or the hybridoma cell line 12.12; (6) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the amino acid sequence shown in SEQ ID NO:10 or SEQ ID NO:12; (7) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay; and (8) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 or CHIR-5.9 monoclonal antibody or the foregoing monoclonal antibodies in preceding items (1)-(7), where the fragment retains the capability of specifically binding to the human CD40 antigen.

### Anti-CD20 Antibodies

By “CD20 antigen” is intended a 33-37 kDa non-glycosylated transmembrane protein that is expressed on lineage-committed B cells from the pre-B cell stage to the B cell lymphoblast stage (GenBank Accession No. X12530; Barclay *et al.* (1997) *The Leucocyte Antigen Facts Book* (2d ed.; Academic Press, San Diego). The CD20 receptor is displayed on the surface of B cell types, as described elsewhere herein. By “displayed on the surface” and “expressed on the surface” is intended that all or a portion of the CD20 antigen is exposed to the exterior of the cell.

Anti-CD20 antibodies are known in the art. See, for example, U.S. Patent Nos. 5,595,721, 6,399,061, and 6,455,043. Human and chimeric anti-CD20 antibodies are particularly useful in the practice of the methods of the invention. Examples of chimeric anti-CD20 antibodies include, but are not limited to, IDEC-C2B8, available commercially under the name Rituxan<sup>®</sup> (IDEC Pharmaceuticals Corp., San Diego, Calif.) and described in U.S. Patent Nos. 5,736,137, 5,776,456, and 5,843,439; the chimeric antibodies described in U.S. Patent No. 5,750,105; and those antibodies described in U.S. Patent Nos. 5,500,362; 5,677,180; 5,721,108; and 5,843,685; the contents of each of which are herein incorporated by reference in their entirety. Anti-CD20 antibodies of murine origin are also suitable for use in the methods of the present invention. Examples of such murine anti-CD20 antibodies include, but are not limited to, the B1 antibody (described in U.S. Patent No. 6,015,542); the IF5 antibody (see Press *et al.* (1989) *J. Clin. Oncol.* 7:1027); NKI-B20 and BCA-B20 anti-CD20 antibodies (described in Hooijberg *et al.* (1995) *Cancer Research* 55:840-846); and IDEC-2B8 (available commercially from IDEC Pharmaceuticals Corp., San Diego, Calif.); the 2H7 antibody (described in Clark *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:1766-1770; and others described in Clark *et al.* (1985) *supra* and Stashenko *et al.* (1980) *J. Immunol.* 125:1678-1685.

### Production of Anti-CD20 and Anti-CD40 Antibodies

The anti-CD40 antibodies and anti-CD20 antibodies for use in the methods of the present invention can be produced using any of the methods well known to those of skill in the art. Polyclonal sera may be prepared by conventional methods. In general, a solution containing the CD40 or the CD20 antigen is first used to immunize a suitable

animal, preferably a mouse, rat, rabbit, or goat. Rabbits or goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies.

5 Polyclonal sera can be prepared in a transgenic animal, preferably a mouse bearing human immunoglobulin loci. In a preferred embodiment, Sf9 cells expressing CD40 or CD20 are used as the immunogen. Immunization can also be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically  
10 sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera are obtained by bleeding the immunized animal into a  
15 glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000 x g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Production of the Sf 9 (*Spodoptera frugiperda*) cells is disclosed in U.S. Patent No. 6,004,552, incorporated herein by reference. Briefly, sequences encoding human  
20 CD40 were recombined into a baculovirus using transfer vectors. The plasmids were co-transfected with wild-type baculovirus DNA into Sf 9 cells. Recombinant baculovirus-infected Sf 9 cells were identified and clonally purified.

Preferably the antibody is monoclonal in nature. Monoclonal antibodies are highly specific, being directed against a single antigenic site, i.e., the CD40 or CD20 cell  
25 surface antigen. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies,  
30 such as those produced by a clonal population of B cells, and is not to be construed as requiring production of the antibody by any particular method. For example, the

monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in, for example, Clackson *et al.* (1991) *Nature* 352:624-628; Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597; and U.S. Patent No. 5,514,548.

By “epitope” is intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind. Epitopes can comprise linear amino acid residues (*i.e.*, residues within the epitope are arranged sequentially one after another in a linear fashion), nonlinear amino acid residues (referred to herein as “nonlinear epitopes”; these epitopes are not arranged sequentially), or both linear and nonlinear amino acid residues.

Monoclonal antibodies can be prepared using the method of Kohler *et al.* (1975) *Nature* 256:495-496, or a modification thereof. Typically, a mouse is immunized with a solution containing an antigen. Immunization can be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund’s complete adjuvant, and injecting the mixture or emulsion parenterally. Any method of immunization known in the art may be used to obtain the monoclonal antibodies of the invention. After immunization of the animal, the spleen (and optionally, several large lymph nodes) are removed and dissociated into single cells. The spleen cells may be screened by applying a cell suspension to a plate or well coated with the antigen of interest. The B cells expressing membrane bound immunoglobulin specific for the antigen bind to the plate and are not rinsed away. Resulting B cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium. The resulting cells are plated by serial dilution and are assayed for the production of antibodies that specifically bind the antigen of interest (and that do not bind to unrelated antigens). The selected monoclonal antibody (mAb)-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

Where the antagonist anti-CD40 antibodies of the invention are to be prepared using recombinant DNA methods, the DNA encoding the monoclonal antibodies is



readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells described herein serve as a preferred source of such DNA. Once isolated, the DNA may be placed into  
5 expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.* (1993) *Curr. Opinion in Immunol.* 5:256 and  
10 Phickthun (1992) *Immunol. Revs.* 130:151. As an alternative to the use of hybridomas, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Patent Nos. 5,545,403; 5,545,405; and 5,998,144; incorporated herein by reference. Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric  
15 antibodies can be produced. Another advantage is the correct glycosylation of the antibody.

In some embodiments, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof is produced in CHO cells using the GS gene expression system (Lonza Biologics, Portsmouth, New  
20 Hampshire), which uses glutamine synthetase as a marker. See, also U.S. Patent Nos. 5,122,464; 5,591,639; 5,658,759; 5,770,359; 5,827,739; 5,879,936; 5,891,693; and 5,981,216; the contents of which are herein incorporated by reference in their entirety.

The term "antigen epitope" as used herein refers to a three dimensional molecular structure (either linear or conformational) that is capable of immunoreactivity with an  
25 anti-CD40 monoclonal antibody or an anti-CD20 monoclonal antibody. Antigen epitopes may comprise proteins, protein fragments, peptides, carbohydrates, lipids, and other molecules, but for the purposes of the present invention are most commonly proteins, short oligopeptides, oligopeptide mimics (i.e., organic compounds that mimic the antibody binding properties of the CD40 or CD20 antigen), or combinations thereof.  
30 Suitable oligopeptide mimics are described, *inter alia*, in PCT application US 91/04282.

Additionally, the term “antibody” as used herein encompasses chimeric anti-CD40 or anti-CD20 antibodies. Chimeric anti-CD40 antibodies for use in the methods of the invention have the binding characteristics of the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9, while chimeric anti-CD20 antibodies for use in the methods of the invention have the binding characteristics of the anti-CD20 monoclonal antibody IDEC-C2B8. By “chimeric” antibodies is intended antibodies that are most preferably derived using recombinant deoxyribonucleic acid techniques and which comprise both human (including immunologically “related” species, e.g., chimpanzee) and non-human components. Thus, the constant region of the chimeric antibody is most preferably substantially identical to the constant region of a natural human antibody; the variable region of the chimeric antibody is most preferably derived from a non-human source and has the desired antigenic specificity to the CD40 or CD20 cell-surface antigen. The non-human source can be any vertebrate source that can be used to generate antibodies to a human CD40 or CD20 cell-surface antigen or material comprising a human CD40 or CD20 cell-surface antigen. Such non-human sources include, but are not limited to, rodents (e.g., rabbit, rat, mouse, etc.; see, for example, U.S. Patent No. 4,816,567, herein incorporated by reference) and non-human primates (e.g., Old World Monkey, Ape, etc.; see, for example, U.S. Patent Nos. 5,750,105 and 5,756,096; herein incorporated by reference). As used herein, the phrase “immunologically active” when used in reference to chimeric anti-CD40 antibodies means a chimeric antibody that binds human CD40, or, when used in reference to chimeric anti-CD20 antibodies means a chimeric antibody that binds human CD20.

Humanized anti-CD40 and anti-CD20 antibodies represent additional anti-CD40 antibodies and anti-CD20 antibodies suitable for use in the methods of the present invention. By “humanized” is intended forms of anti-CD40 antibodies or anti-CD20 antibodies that contain minimal sequence derived from non-human immunoglobulin sequences. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (also known as complementarity determining region or CDR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. The

phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. See, e.g., Chothia *et al* ( 1987) *J. Mol. Biol.* 196:901-917; Kabat *et al* (1991) U. S. Dept. of Health and Human Services, NIH Publication No. 91-3242). The phrase "constant region" refers to the portion of the antibody molecule that confers effector functions. In previous work directed towards producing non-immunogenic antibodies for use in therapy of human disease, mouse constant regions were substituted by human constant regions. The constant regions of the subject humanized antibodies were derived from human immunoglobulins. However, these humanized antibodies still elicited an unwanted and potentially dangerous immune response in humans and there was a loss of affinity. Humanized anti-CD40 antibodies for use in the methods of the present invention have binding characteristics similar to those exhibited by the CHIR-5.9 and CHIR-12.12 monoclonal antibodies described herein. Humanized anti-CD20 antibodies for use in the methods of the present invention have binding characteristics similar to those exhibited by IDEC-C2B8 monoclonal antibodies described herein.

Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeven *et al.* (1988) *Science* 239:1534-1536), by substituting rodent or mutant rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. See also U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205; herein incorporated by reference. In some instances, residues within the framework regions of one or more variable regions of the human immunoglobulin are replaced by corresponding non-human residues (see, for example, U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370). Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (e.g., to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin

sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.* (1986) *Nature* 331:522-525; Riechmann *et al.* (1988) *Nature* 332:323-329; and Presta (1992) *Curr. Op. Struct. Biol.* 2:593-596; herein  
5 incorporated by reference. Accordingly, such "humanized" antibodies may include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from analogous sites in  
10 rodent antibodies. See, for example, U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Patent No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.

Also encompassed by the term anti-CD40 antibodies or anti-CD20 antibodies are  
15 xenogenic or modified anti-CD40 antibodies or anti-CD20 antibodies produced in a non-human mammalian host, more particularly a transgenic mouse, characterized by inactivated endogenous immunoglobulin (Ig) loci. In such transgenic animals, competent endogenous genes for the expression of light and heavy subunits of host immunoglobulins are rendered non-functional and substituted with the analogous human  
20 immunoglobulin loci. These transgenic animals produce human antibodies in the substantial absence of light or heavy host immunoglobulin subunits. See, for example, U.S. Patent Nos. 5,877,397 and 5,939,598, herein incorporated by reference.

Preferably, fully human antibodies to CD40 or CD20 are obtained by immunizing transgenic mice. One such mouse is obtained using XenoMouse<sup>®</sup> technology (Abgenix;  
25 Fremont, California), and is disclosed in U.S. Patent Nos. 6,075,181, 6,091,001, and 6,114,598, all of which are incorporated herein by reference. To produce the antibodies disclosed herein, mice transgenic for the human Ig G<sub>1</sub> heavy chain locus and the human  $\kappa$  light chain locus can be immunized with Sf 9 cells expressing human CD40 or human CD20. Mice can also be transgenic for other isotypes. Fully human antibodies useful in  
30 the methods of the present invention are characterized by binding properties similar to those exhibited by the CHIR-5.9, CHIR-12.12, and IDEC-C2B8 monoclonal antibodies.

Fragments of the anti-CD40 antibodies or anti-CD20 antibodies are suitable for use in the methods of the invention so long as they retain the desired affinity of the full-length antibody. Thus, a fragment of an anti-CD40 antibody will retain the ability to bind to the CD40 B cell surface antigen, and a fragment of an anti-CD20 antibody will retain the ability to bind the CD20 B cell surface antigen, respectively. Such fragments are characterized by properties similar to the corresponding full-length antibody. For example, antagonist anti-CD40 antibody fragments will specifically bind a human CD40 antigen expressed on the surface of a human cell, and are free of significant agonist activity but exhibit antagonist activity when bound to a CD40 antigen on a human CD40-expressing cell; whereas anti-CD20 antibody fragments will specifically bind CD20. Such fragments are referred to herein as “antigen-binding” fragments.

Suitable antigen-binding fragments of an antibody comprise a portion of a full-length antibody, generally the antigen-binding or variable region thereof. Examples of antibody fragments include, but are not limited to, Fab, F(ab')<sub>2</sub>, and Fv fragments and single-chain antibody molecules. By “Fab” is intended a monovalent antigen-binding fragment of an immunoglobulin that is composed of the light chain and part of the heavy chain. By F(ab')<sub>2</sub> is intended a bivalent antigen-binding fragment of an immunoglobulin that contains both light chains and part of both heavy chains. By “single-chain Fv” or “sFv” antibody fragments is intended fragments comprising the V<sub>H</sub> and V<sub>L</sub> domains of an antibody, wherein these domains are present in a single polypeptide chain. See, for example, U.S. Patent Nos. 4,946,778, 5,260,203, 5,455,030, and 5,856,456, herein incorporated by reference. Generally, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains that enables the sFv to form the desired structure for antigen-binding. For a review of sFv see Pluckthun (1994) in *The Pharmacology of Monoclonal Antibodies*, Vol. 113, ed. Rosenberg and Moore (Springer-Verlag, New York), pp. 269-315.

Antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in, for example, McCafferty *et al.* (1990) *Nature* 348:552-554 (1990) and U.S. Patent No. 5,514,548. Clackson *et al.* (1991) *Nature* 352:624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597 describe the isolation of murine and human antibodies, respectively, using phage libraries.

Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.* (1992) *Bio/Technology* 10:779-783), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.* (1993) *Nucleic. Acids Res.* 21:2265-2266). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived *via* proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.* (1992) *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.* (1985) *Science* 229:81). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')<sub>2</sub> fragments (Carter *et al.* (1992) *Bio/Technology* 10:163-167).

According to another approach, F(ab')<sub>2</sub> fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

Combinations of antibodies useful in the methods of the present invention include antagonist anti-CD40 antibodies such as the CHIR-5.9 and CHIR-12.12 monoclonal antibodies disclosed herein as well as anti-CD20 antibodies such as IDEC-C2B8 that differ in non-CDR regions; and antibodies with one or more amino acid addition(s), deletion(s), or substitution(s). The invention also encompasses de-immunized (humanized) anti-CD20 antibodies and antagonist anti-CD40 antibodies, which can be produced as described in, for example, International Publication Nos. WO 98/52976 and WO 0034317; herein incorporated by reference. In this manner, residues within the antibodies useful for the practicing the methods of the invention are modified so as to render the antibodies non- or less immunogenic to humans while retaining their binding specificity and biological activity, wherein such activity is measured by assays noted elsewhere herein. Also included within the scope of the claims are fusion proteins comprising anti CD20 antibodies or antagonist anti-CD40 antibodies, or a fragment thereof, which fusion proteins can be synthesized or expressed from corresponding

polynucleotide vectors, as is known in the art. Such fusion proteins are described with reference to conjugation of antibodies as noted below.

The antibodies useful in practicing the methods of the invention can have sequence variations produced using methods described in, for example, Patent  
5 Publication Nos. EP 0 983 303 A1, WO 00/34317, and WO 98/52976, incorporated herein by reference. For example, it has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T-cell response. A conservative substitution can allow the antibody to retain binding activity yet  
10 lose its ability to trigger an unwanted T-cell response. Any such conservative or non-conservative substitutions can be made using art-recognized methods, such as those noted elsewhere herein, and the resulting antibodies will fall within the scope of the invention. The variant antibodies can be routinely tested for antagonist activity, affinity, and specificity using methods described herein.

An antagonistic anti-CD40 antibody produced by any of the methods described  
15 above, or any other method not disclosed herein, will fall within the scope of the invention if it possesses at least one of the following biological activities: inhibition of immunoglobulin secretion by normal human peripheral B cells stimulated by T cells; inhibition of proliferation of normal human peripheral B cells stimulated by Jurkat T cells; inhibition of proliferation of normal human peripheral B cells stimulated by  
20 CD40L-expressing cells or soluble CD40; and inhibition of proliferation of human malignant B cells as noted below. These assays can be performed as described in copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney  
25 Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety. See also the assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15;  
30 Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998) *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991) *Current*

*Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082; herein incorporated by reference.

A representative assay to detect antagonistic anti-CD40 antibodies specific to the CD40-antigen epitopes identified herein or is a "competitive binding assay".

5 Competitive binding assays are serological assays in which unknowns are detected and quantitated by their ability to inhibit the binding of a labeled known ligand to its specific antibody. This is also referred to as a competitive inhibition assay. In a representative competitive binding assay, labeled CD40 polypeptide is precipitated by candidate antibodies in a sample, for example, in combination with monoclonal antibodies raised  
10 against one or more epitopes of the monoclonal antibodies of the invention. Anti-CD40 antibodies that specifically react with an epitope of interest can be identified by screening a series of antibodies prepared against a CD40 protein or fragment of the protein comprising the particular epitope of the CD40 protein of interest. For example, for human CD40, epitopes of interest include epitopes comprising linear and/or nonlinear  
15 amino acid residues of the short isoform of human CD40 (see GenBank Accession No. NP\_690593) set forth in Figure 5B (SEQ ID NO:10), encoded by the sequence set forth in Figure 5A (SEQ ID NO:9; see also GenBank Accession No. NM\_152854), or of the long isoform of human CD40 (see GenBank Accession Nos. CAA43045 and NP\_001241) set forth in Figure 5D (SEQ ID NO:12), encoded by the sequence set forth  
20 in Figure 5C (SEQ ID NO:11; see GenBank Accession Nos. X60592 and NM\_001250). Alternatively, competitive binding assays with previously identified suitable antagonist anti-CD40 antibodies could be used to select monoclonal antibodies comparable to the previously identified antibodies.

An anti-CD20 antibody produced by any of the methods described above, or any  
25 other method not disclosed herein, will fall within the scope of the invention if it possesses at least one of the following biological activities: initiation of antibody-dependent cell-mediated cytotoxicity against a CD20 expressing cell; initiation of complement-mediated cytotoxicity against a CD20 expressing cell; delivery of a cytotoxin or radionuclide to a CD20 expressing cell; inhibition of immunoglobulin  
30 secretion by normal human peripheral B cells stimulated by T cells; inhibition of proliferation of normal human peripheral B cells stimulated by Jurkat T cells; inhibition



of proliferation of normal human peripheral B cells stimulated by CD40L-expressing cells or soluble CD40; and inhibition of proliferation of human malignant B cells as noted below. Assays for detecting these activities are well known in the art, including those disclosed in U.S. Patent No. 5,736,137, herein incorporated by reference in its entirety.

5 Any of the previously described antagonist anti-CD40 antibodies (or antigen-binding fragments thereof) or anti-CD20 antibodies (or antigen-binding fragments thereof) may be conjugated prior to use in the methods of the present invention. Methods for producing conjugated antibodies are known in the art. Thus, the anti-CD40 antibody or anti-CD20 antibody may be labeled using an indirect labeling or indirect labeling  
10 approach. By “indirect labeling” or “indirect labeling approach” is intended that a chelating agent is covalently attached to an antibody and at least one radionuclide is inserted into the chelating agent. See, for example, the chelating agents and radionuclides described in Srivastava and Mease (1991) *Nucl. Med. Bio.* 18:589-603, herein incorporated by reference. Suitable labels include fluorophores, chromophores,  
15 radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. “Specific binding partner” refers to a protein capable of binding a  
20 ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefore. Other specific binding partners include biotin and avidin or streptavidin, Ig G and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several  
25 different modes. For example,  $^{125}\text{I}$  may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a mAb. Further, one may combine various labels for desired effect. For example, mAbs and avidin also require labels in the practice of this invention: thus, one might label a mAb with biotin, and detect its presence with avidin labeled with  $^{125}\text{I}$ , or with an anti-biotin mAb labeled with  
30 HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Alternatively, the anti-CD40 antibody or anti-CD20 antibody may be labeled using "direct labeling" or a "direct labeling approach," where a radionuclide is covalently attached directly to an antibody (typically via an amino acid residue). Preferred radionuclides are provided in Srivastava and Mease (1991) *supra*. The indirect labeling approach is particularly preferred. See also, for example, International Publication Nos. WO 00/52031 and WO 00/52473, where a linker is used to attach a radioactive label to antibodies; and the labeled forms of antibodies described in U.S. Patent No. 6,015,542; herein incorporated by reference.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive metal ion or radioisotope. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., fludarabine, 2-chlorodeoxyadenosine, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine). Radioisotopes include, but are not limited to, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, Bi-213, Pd-109, Tc-99, In-111, and the like. The conjugates of the invention can be used for modifying a given biological response; the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, interferon-alpha, interferon-beta, nerve

growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or  
5 other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon *et al.* (1985) "Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy," in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld *et al.* (Alan R. Liss, Inc.), pp. 243-256; ed. Hellstrom *et al.* (1987) "Antibodies for Drug  
10 Delivery," in *Controlled Drug Delivery*, ed. Robinson *et al.* (2d ed; Marcel Dekker, Inc.), pp. 623-653; Thorpe (1985) "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological and Clinical Applications*, ed. Pinchera *et al.* pp. 475-506 (Editrice Kurtis, Milano, Italy, 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy,"  
15 in *Monoclonal Antibodies for Cancer Detection and Therapy*, ed. Baldwin *et al.* (Academic Press, New York, 1985), pp. 303-316; and Thorpe *et al.* (1982) *Immunol. Rev.* 62:119-158.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in U.S. Patent No. 4,676,980. In addition, linkers  
20 may be used between the labels and the antibodies of the invention (see U.S. Patent No. 4,831,175). Antibodies or, antigen-binding fragments thereof may be directly labeled with radioactive iodine, indium, yttrium, or other radioactive particle known in the art (U.S. Patent No. 5,595,721). Treatment may consist of a combination of treatment with conjugated and nonconjugated antibodies administered simultaneously or sequentially, in  
25 either order, on the same or different days (WO 00/52031 and WO 00/52473). In some embodiments, the anti-CD20 antibody is conjugated to the anti-CD40 antibody. In yet other embodiments, a single antibody comprises dual specificity toward both CD20 and CD40. Such bispecific antibodies are known in the art. See, for example, U.S. Patent No. 5,959,084.

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Variants of Antagonist Anti-CD40 Antibodies and Anti-CD20 Antibodies

Suitable biologically active variants of the antagonist anti-CD40 antibodies or anti-CD20 antibodies can be used in the methods of the present invention. Such variants will retain the desired binding properties of the parent antibody, i.e., the parent antagonist anti-CD40 antibody or parent anti-CD20 antibody. Methods for making antibody  
5 variants are generally available in the art.

For example, amino acid sequence variants of an anti-CD20 antibody, for example IDEC-C2B8 described herein, or an antagonist anti-CD40 antibody, for example, the CHIR-5.9 or CHIR-12.12 monoclonal antibody described herein, can be prepared by mutations in the cloned DNA sequence encoding the antibody of interest.

10 Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods Enzymol.* 154:367-382; Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York); U.S.  
15 Patent No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest may be found in the model of Dayhoff *et al.* (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one  
20 amino acid with another having similar properties, may be preferred. Examples of conservative substitutions include, but are not limited to, Gly $\leftrightarrow$ Ala, Val $\leftrightarrow$ Ile $\leftrightarrow$ Leu, Asp $\leftrightarrow$ Glu, Lys $\leftrightarrow$ Arg, Asn $\leftrightarrow$ Gln, and Phe $\leftrightarrow$ Trp $\leftrightarrow$ Tyr.

In constructing variants of the anti-CD-20 antibody or antagonist anti-CD40 antibody polypeptide of interest, modifications are made such that variants continue to  
25 possess the desired activity, i.e., similar binding affinity and are capable of specifically binding to a human CD20 or CD40 antigen expressed on the surface of a human cell, respectively, and in the case of anti-CD40 being free of significant agonist activity but exhibiting antagonist activity when bound to a CD40 antigen on a human CD40-expressing cell. Obviously, any mutations made in the DNA encoding the variant  
30 polypeptide must not place the sequence out of reading frame and preferably will not

create complementary regions that could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

In addition, the constant region of an antagonist anti-CD40 antibody can be mutated to alter effector function in a number of ways. For example, see U.S. Patent No. 6,737,056B1 and U.S. Patent Application Publication No. 2004/0132101A1, which disclose Fc mutations that optimize antibody binding to Fc receptors.

Preferably, variants of a reference anti-CD20 antibody or antagonist anti-CD40 antibody have amino acid sequences that have at least 70% or 75% sequence identity, preferably at least 80% or 85% sequence identity, more preferably at least 90%, 91%, 92%, 93%, 94% or 95% sequence identity to the amino acid sequence for the reference anti-CD20 antibody, for example IDEC-C2B8 as described herein, or reference antagonist anti-CD40 antibody molecule, for example, the CHIR-5.9 or CHIR-12.12 monoclonal antibody described herein, or to a shorter portion of the reference antibody molecule. More preferably, the molecules share at least 96%, 97%, 98% or 99% sequence identity. For purposes of the present invention, percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489. A variant may, for example, differ from the reference antagonist anti-CD40 antibody or reference anti-CD20 antibody by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for sequence identity associated with conservative residue substitutions or gaps can be made (see Smith-Waterman homology search algorithm).

The precise chemical structure of a polypeptide capable of specifically binding CD40 and retaining antagonist activity or a polypeptide specifically binding CD20,

particularly when bound to antigen on malignant B cells, depends on a number of factors. As ionizable amino and carboxyl groups are present in the molecule, a particular polypeptide may be obtained as an acidic or basic salt, or in neutral form. All such preparations that retain their biological activity when placed in suitable environmental conditions are included in the definition of antagonist anti-CD40 antibodies or anti-CD20 antibodies as used herein. Further, the primary amino acid sequence of the polypeptide may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like. It may also be augmented by conjugation with saccharides. Certain aspects of such augmentation are accomplished through post-translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In any event, such modifications are included in the definition of an anti-CD40 antibody or an anti-CD20 antibody used herein so long as the binding properties of the anti-CD40 antibody (including antagonist activity) or anti-CD20 antibody are not destroyed. It is expected that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the polypeptide, in the various assays. Further, individual amino acid residues in the chain may be modified by oxidation, reduction, or other derivatization, and the polypeptide may be cleaved to obtain fragments that retain activity. Such alterations that do not destroy the desirable activity of the unmodified antibody do not remove the polypeptide sequence from the definition of the anti-CD40 and anti-CD20 antibodies of interest as used herein.

The art provides substantial guidance regarding the preparation and use of polypeptide variants. In preparing the antibody variants, one of skill in the art can readily determine which modifications to the native protein nucleotide or amino acid sequence will result in a variant that is suitable for use as a therapeutically active component of a pharmaceutical composition used in the methods of the present invention.

#### Pharmaceutical Formulations and Modes of Administration

The combination of the anti-CD20 antibody (or antigen-binding fragment thereof) and the antagonist anti-CD40 antibody (or antigen-binding fragment thereof) is administered at a concentration that is therapeutically effective to prevent or treat a

cancer characterized by neoplastic B cell growth, particularly cancers comprising neoplastic B cells expressing both the CD40 and CD20 antigens. To accomplish this goal, the antibodies may be formulated using a variety of acceptable excipients known in the art. Typically, the antibodies are administered by injection, either intravenously, 5 intraperitoneally, or subcutaneously. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes.

Intravenous administration occurs preferably by infusion over a period of about 1 10 to about 10 hours, more preferably over about 1 to about 8 hours, even more preferably over about 2 to about 7 hours, still more preferably over about 4 to about 6 hours, depending upon the antibody being administered. The initial infusion with the pharmaceutical composition may be given over a period of about 4 to about 6 hours with subsequent infusions delivered more quickly. Subsequent infusions may be administered 15 over a period of about 1 to about 6 hours, including, for example, about 1 to about 4 hours, about 1 to about 3 hours, or about 1 to about 2 hours.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of possible routes of administration include parenteral, (e.g., intravenous (IV), intramuscular (IM), intradermal, subcutaneous 20 (SC), or infusion), oral and pulmonary (e.g., inhalation), nasal, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl 25 alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, 30 or multiple dose vials made of glass or plastic.

The antibodies are typically provided by standard technique within a pharmaceutically acceptable buffer; for example, sterile saline, sterile buffered water, propylene glycol, combinations of the foregoing, etc. The antagonist anti-CD40 antibody (or antigen-binding fragment thereof) and the anti-CD20 antibody (or antigen-binding  
5 fragment thereof) can be formulated in separate pharmaceutical compositions, or can be formulated within a single pharmaceutical composition for simultaneous administration. Methods for preparing parenterally administrable agents are described in *Remington's Pharmaceutical Sciences* (18<sup>th</sup> ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference. See, also, for example, WO 98/56418, which  
10 describes stabilized antibody pharmaceutical formulations suitable for use in the methods of the present invention.

The amount of a combination of at least one anti-CD40 antibody or antigen-binding fragment thereof and at least one anti-CD20 antibody or antigen-binding fragment thereof to be administered is readily determined by one of ordinary skill in the  
15 art without undue experimentation. Factors influencing the mode of administration and the respective amount of the combination of antibodies disclosed herein include, but are not limited to, the particular disease undergoing therapy, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of the combination of antibodies  
20 disclosed herein to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of these anti-tumor agents. Generally, a higher dosage of the combination of antibodies disclosed herein is preferred with increasing weight of the patient undergoing therapy. The dose of either the anti-CD20 antibody (or antigen-binding fragment thereof) or the antagonistic anti-  
25 CD40 antibody (or antigen-binding fragment thereof) to be administered is in the range from about 0.003 mg/kg to about 50 mg/kg, preferably in the range of 0.01 mg/kg to about 40 mg/kg. Thus, for example, the dose of any one antibody of the combination can be 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2  
mg/kg, 2.5 mg/kg, 3 mg/kg, 5 mg/kg, 7 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25  
30 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, or 50 mg/kg.



In another embodiment of the invention, the method comprises administration of multiple doses of anti-CD20 antibody (or antigen-binding fragment thereof) in combination with multiple doses of antagonistic anti-CD40 antibody (or antigen-binding fragment thereof). The method may comprise administration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, or more therapeutically effective doses of a pharmaceutical composition comprising either anti-CD20 antibody (or antigen-binding fragment thereof) or antagonistic anti-CD40 antibody (or antigen-binding fragment thereof), or both. The frequency and duration of administration of multiple doses of the pharmaceutical compositions can be readily determined by one of skill in the art without undue experimentation. Moreover, treatment of a subject with a therapeutically effective amount of a combination of antibodies can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with the combination of an anti-CD20 antibody (or antigen-binding fragment thereof) and an antagonistic anti-CD40 antibody (or antigen-binding fragment thereof), where both are administered at a dose in the range of between about 0.1 to about 20 mg/kg body weight, once per week for between about 1 to about 10 weeks, preferably between about 2 to about 8 weeks, more preferably between about 3 to about 7 weeks, and even more preferably for about 4, 5, or 6 weeks. Treatment may occur annually to prevent relapse or upon indication of relapse.

It will also be appreciated that the effective dosage of antibodies or antigen-binding fragments thereof used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein. Thus, in one embodiment, the dosing regimen includes administration of a therapeutically effective dose of the anti-CD20 antibody (or antigen-binding fragment thereof) in combination with a therapeutically effective dose of the antagonistic anti-CD40 antibody (or antigen-binding fragment thereof), where the combination is administered on days 1, 8, 15, and 22 of a treatment period. In another embodiment, the dosing regimen includes administration of a therapeutically effective dose of the anti-CD20 antibody (or antigen-binding fragment thereof) in combination with a therapeutically effective dose of the antagonist anti-CD40 antibody (or antigen-binding fragment thereof), where the combination is administered

on days 1, 2, 3, 4, 5, 6, and 7 of a week in a treatment period. Further embodiments include a dosing regimen where a therapeutically effective dose of the anti-CD20 antibody (or antigen-binding fragment thereof) is administered in combination with a therapeutically effective dose of the antagonist anti-CD40 antibody (or antigen-binding fragments thereof), where the combination is administered on days 1, 3, 5, and 7 of a week in a treatment period; a dosing regimen that includes administration of a therapeutically effective dose of the anti-CD20 antibody (or antigen-binding fragment thereof) in combination with a therapeutically effective dose of the antagonist anti-CD40 antibody (or antigen-binding fragment thereof), where the combination of antibodies is administered on days 1 and 3 of a week in a treatment period; and a preferred dosing regimen that includes administration of a therapeutically effective dose of the anti-CD20 antibody (or antigen-binding fragment thereof) in combination with the antagonist anti-CD40 antibody (or antigen-binding fragments thereof) on day 1 of any given week in a treatment period. The treatment period may comprise 1 week, 2 weeks, 3 weeks, a month, 3 months, 6 months, or a year. Treatment periods may be subsequent or separated from each other by a day, a week, 2 weeks, a month, 3 months, 6 months, or a year. Treatment using a combination of antagonist anti-CD40 antibody (or antigen-binding fragment thereof) and anti-CD20 antibody (or antigen-binding fragment thereof) may comprise administration of one or both antibodies simultaneously or concurrently, as long as the treatment includes the combination of anti-CD20 antibody (or antigen-binding fragment thereof) and antagonist anti-CD40 antibody (or antigen-binding fragment thereof) at some point during treatment. The effect of the combination therapy can also be optimized by varying the timing of administration of either the anti-CD20 antibody and/or the antagonist anti-CD40 antibody treatment. Treatment with an anti-CD20 antibody or antigen-binding fragment thereof in combination with an antagonist anti-CD40 antibody or antigen-binding fragment thereof can be simultaneous (concurrent), consecutive (sequential), or a combination thereof. Therefore, a subject undergoing combination antibody therapy can receive both the anti-CD20 antibody (or antigen-binding fragment thereof) and antagonist anti-CD40 (or antigen-binding fragment thereof) at the same time (i.e., simultaneously) or at different times (i.e., sequentially, in either order, on the same day, or on different days). Thus, in some embodiments, the

anti-CD20 antibody, such as Rituxan<sup>®</sup> (or antigen-binding fragment thereof) is administered simultaneously with the antagonist anti-CD40 antibody, such as the monoclonal antibody CHIR-12.12. or CHIR-5.9 (or antigen-binding fragment thereof). In other embodiments, the anti-CD20 antibody, such as Rituxan<sup>®</sup> (or antigen-binding fragment thereof) is administered first and then the antagonist anti-CD40 antibody, such as the monoclonal antibody CHIR-12.12. or CHIR-5.9 (or antigen-binding fragment thereof) is administered next. In yet other embodiments, the antagonist anti-CD40 antibody, such as the monoclonal antibody CHIR-12.12 or CHIR-5.9 (or antigen-binding fragment thereof) is administered first, and the anti-CD20 antibody, such as Rituxan<sup>®</sup> (or antigen-binding fragment thereof) is administered next. In some embodiments, the combination of anti-CD20 antibodies and antagonist anti-CD40 antibodies, such as Rituxan<sup>®</sup> and monoclonal antibodies CHIR-12.12 or CHIR-5.9, is given concurrently for one dosing, but other dosings include sequential administration, in either order, on the same day, or on different days. Where the anti-CD20 antibody such as Rituxan<sup>®</sup> and the antagonist anti-CD40 antibody such as the monoclonal antibody CHIR-12.12 or CHIR-5.9 are administered simultaneously, they can be administered as separate pharmaceutical compositions, each comprising either the anti-CD20 antibody (or antigen-binding fragment thereof) or the antagonist anti-CD40 antibody (or antigen-binding fragment thereof), or can be administered as a single pharmaceutical composition comprising both of these anti-cancer agents.

In some embodiments, the therapeutically effective doses of antagonist anti-CD40 antibody or antigen-binding fragment thereof ranges from about 0.003 mg/kg to about 50 mg/kg, from about 0.01 mg/kg to about 40 mg/kg, from about 0.01 mg/kg to about 30 mg/kg, from about 0.1 mg/kg to about 30 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 25 mg/kg, from about 3 mg/kg to about 20 mg/kg, from about 5 mg/kg to about 15 mg/kg, or from about 7 mg/kg to about 12 mg/kg. Thus, for example, the dose of any one antagonist anti-CD40 antibody or antigen-binding fragment thereof, for example the anti-CD40 monoclonal antibody CHIR-12.12 or CHIR-5.9 or antigen-binding fragment thereof, can be 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 5

mg/kg, 7 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, or other such doses falling within the range of about 0.003 mg/kg to about 50 mg/kg. The same therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof can be administered throughout each week of antibody dosing. Alternatively, different therapeutically effective doses of an antagonist anti-CD40 antibody or antigen-binding fragment thereof can be used over the course of a treatment period.

In other embodiments, the initial therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof as defined elsewhere herein can be in the lower dosing range (i.e., about 0.003 mg/kg to about 20 mg/kg) with subsequent doses falling within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg).

In alternative embodiments, the initial therapeutically effective dose of an antagonist anti-CD40 antibody or antigen-binding fragment thereof as defined elsewhere herein can be in the upper dosing range (i.e., about 20 mg/kg to about 50 mg/kg) with subsequent doses falling within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg). Thus, in one embodiment, the initial therapeutically effective dose of the antagonist anti-CD40 antibody or antigen-binding fragment thereof is about 20 mg/kg to about 35 mg/kg, including about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, and about 35 mg/kg, and subsequent therapeutically effective doses of the antagonist anti-CD40 antibody or antigen binding fragment thereof are about 5 mg/kg to about 15 mg/kg, including about 5 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, and about 15 mg/kg.

In some embodiments of the invention, antagonist anti-CD40 antibody therapy is initiated by administering a "loading dose" of the antibody or antigen-binding fragment thereof to the subject in need of antagonist anti-CD40 antibody therapy. By "loading dose" is intended an initial dose of the antagonist anti-CD40 antibody or antigen-binding fragment thereof that is administered to the subject, where the dose of the antibody or antigen-binding fragment thereof administered falls within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg). The "loading dose" can be administered as a single administration, for example, a single infusion where the antibody or antigen-binding fragment thereof is administered IV, or as multiple administrations, for example,

multiple infusions where the antibody or antigen-binding fragment thereof is administered IV, so long as the complete "loading dose" is administered within about a 24-hour period. Following administration of the "loading dose," the subject is then administered one or more additional therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. Subsequent therapeutically effective doses can be administered, for example, according to a weekly dosing schedule, or once every two weeks, once every three weeks, or once every four weeks. In such embodiments, the subsequent therapeutically effective doses generally fall within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg).

Alternatively, in some embodiments, following the "loading dose," the subsequent therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered according to a "maintenance schedule," wherein the therapeutically effective dose of the antibody or antigen-binding fragment thereof is administered once a month, once every 6 weeks, once every two months, once every 10 weeks, once every three months, once every 14 weeks, once every four months, once every 18 weeks, once every five months, once every 22 weeks, once every six months, once every 7 months, once every 8 months, once every 9 months, once every 10 months, once every 11 months, or once every 12 months. In such embodiments, the therapeutically effective doses of the antagonist anti-CD40 antibody or antigen-binding fragment thereof fall within the lower dosing range (i.e., 0.003 mg/kg to about 20 mg/kg), particularly when the subsequent doses are administered at more frequent intervals, for example, once every two weeks to once every month, or within the higher dosing range (i.e., from about 20 mg/kg to about 50 mg/kg), particularly when the subsequent doses are administered at less frequent intervals, for example, where subsequent doses are administered about one month to about 12 months apart.

The pharmaceutical compositions useful in the methods of the invention may comprise biologically active variants of either anti-CD20 antibody (or antigen-binding fragments thereof) or antagonistic anti-CD40 antibody (or antigen-binding fragments thereof), or both. Such variants should retain the desired biological activity of the native polypeptide such that the pharmaceutical composition comprising the variant polypeptide has the same therapeutic effect as the pharmaceutical composition comprising the native

polypeptide when administered to a subject. That is, the variant antibody will serve as a therapeutically active component in the pharmaceutical composition in a manner similar to that observed for the native antagonist antibody; for example, monoclonal antibody CHIR-5.9 or CHIR-12.12 as expressed by the hybridoma cell line 5.9 or 12.12, and IDEC-C2B8, respectively. Methods are available in the art for determining whether a variant antibody retains the desired biological activity, and hence, serves as a therapeutically active component in the pharmaceutical composition. Biological activity of antibody variants can be measured using assays specifically designed for measuring activity of the native antagonist antibody, including assays described in the present invention.

Any pharmaceutical composition comprising an anti-CD20 antibody having the binding properties described herein, or an antagonist anti-CD40 antibody having the binding properties described herein, as the therapeutically active component can be used in the methods of the invention. Thus, liquid, lyophilized, or spray-dried compositions comprising one or more of the antibodies useful in the practice of the invention may be prepared as an aqueous or nonaqueous solution or suspension for subsequent administration to a subject in accordance with the methods of the invention. Each of these compositions will comprise at least one of the anti-CD20 antibodies (or antigen-binding fragment thereof) or antagonist anti-CD40 antibodies (or antigen-binding fragment thereof) as a therapeutically or prophylactically active component. By “therapeutically or prophylactically active component” is intended the antibody or antigen-binding fragment thereof is specifically incorporated into the composition to bring about a desired therapeutic or prophylactic response with regard to treatment, prevention, or diagnosis of a disease or condition within a subject when the pharmaceutical composition is administered to that subject. Preferably the pharmaceutical compositions comprise appropriate stabilizing agents, bulking agents, or both to minimize problems associated with loss of protein stability and biological activity during preparation and storage.

Formulants may be added to pharmaceutical compositions comprising antibodies useful in the practice of the invention. These formulants may include, but are not limited to, oils, polymers, vitamins, carbohydrates, amine acids, salts, buffers, albumin,

surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or polysaccharides, or water soluble glucans. The saccharides or glucans can include fructose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin,  $\alpha$  and  $\beta$  cyclodextrin, soluble starch, hydroxyethyl starch, and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" is defined as a C<sub>4</sub> to C<sub>8</sub> hydrocarbon having a hydroxyl group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. These sugars or sugar alcohols may be used individually or in combination. The sugar or sugar alcohol concentration is between 1.0% and 7% w/v, more preferably between 2.0% and 6.0% w/v. Preferably, amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers and methods to attach them to peptides, are shown in U.S. Patent Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546, which are all hereby incorporated by reference in their entireties. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: R(O--CH<sub>2</sub>--CH<sub>2</sub>)<sub>n</sub>O--R where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1,000 and 40,000, more preferably between 2,000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water-soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), and the like. POG is preferred. One reason is because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in Knauf *et al.* (1988) *J. Bio. Chem.* 263:15064-15070, and a discussion of POG/IL-2 conjugates is found in U.S. Patent No. 4,766,106, both of which are hereby incorporated by reference in their entireties.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in Gabizon *et al.* (1982) *Cancer Research* 42:4734; Cafiso (1981) *Biochem Biophys Acta* 649:129; and Szoka (1980) *Ann. Rev. Biophys. Eng.* 9:467. Other drug delivery systems are known in the art and are described in, *e.g.*, Poznansky *et al.* (1980) *Drug Delivery Systems* (R.L. Juliano, ed., Oxford, N.Y.) pp. 253-315; Poznansky (1984) *Pharm Revs* 36:277.

The formulants to be incorporated into a pharmaceutical composition should provide for the stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. That is, the antagonist anti-CD40 antibody or antigen-binding fragment thereof should retain its physical and/or chemical stability and have the desired biological activity, *i.e.*, one or more of the antagonist activities defined herein above, including, but not limited to, inhibition of immunoglobulin secretion by normal human peripheral B cells stimulated by T cells; inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by Jurkat T cells; inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by CD40L-expressing cells or soluble CD40 ligand (sCD40L); inhibition of "survival" anti-apoptotic intracellular signals in any cell stimulated by sCD40L or solid-phase CD40L; inhibition of CD40 signal transduction in any cell upon ligation with sCD40L or solid-phase CD40L; and inhibition of proliferation of human malignant B cells as noted elsewhere herein.

Methods for monitoring protein stability are well known in the art. See, for example, Jones (1993) *Adv. Drug Delivery Rev.* 10:29-90; Lee, ed. (1991) *Peptide and*



*Protein Drug Delivery* (Marcel Dekker, Inc., New York, New York); and the stability assays disclosed herein below. Generally, protein stability is measured at a chosen temperature for a specified period of time. In preferred embodiments, a stable antibody pharmaceutical formulation provides for stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof when stored at room temperature (about 25°C) for at least 1 month, at least 3 months, or at least 6 months, and/or is stable at about 2-8°C for at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months.

A protein such as an antibody, when formulated in a pharmaceutical composition, is considered to retain its physical stability at a given point in time if it shows no visual signs (i.e., discoloration or loss of clarity) or measurable signs (for example, using size-exclusion chromatography (SEC) or UV light scattering) of precipitation, aggregation, and/or denaturation in that pharmaceutical composition. With respect to chemical stability, a protein such as an antibody, when formulated in a pharmaceutical composition, is considered to retain its chemical stability at a given point in time if measurements of chemical stability are indicative that the protein (i.e., antibody) retains the biological activity of interest in that pharmaceutical composition. Methods for monitoring changes in chemical stability are well known in the art and include, but are not limited to, methods to detect chemically altered forms of the protein such as result from clipping, using, for example, SDS-PAGE, SEC, and/or matrix-assisted laser desorption ionization/time of flight mass spectrometry; and degradation associated with changes in molecular charge (for example, associated with deamidation), using, for example, ion-exchange chromatography. See, for example, the methods disclosed herein below.

An antagonist anti-CD40 antibody or antigen-binding fragment thereof, when formulated in a pharmaceutical composition, is considered to retain a desired biological activity at a given point in time if the desired biological activity at that time is within about 30%, preferably within about 20% of the desired biological activity exhibited at the time the pharmaceutical composition was prepared as determined in a suitable assay for the desired biological activity. Assays for measuring the desired biological activity of the antagonist anti-CD40 antibodies disclosed herein, and antigen-binding fragments thereof,

can be performed as described in the Examples herein. See also the assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15; Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998) *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991) *Current Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082; herein incorporated by reference.

In some embodiments of the invention, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof is formulated in a liquid pharmaceutical formulation. The antagonist anti-CD40 antibody or antigen binding fragment thereof can be prepared using any method known in the art, including those methods disclosed herein above. In one embodiment, the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof is recombinantly produced in a CHO cell line.

Following its preparation and purification, the antagonist anti-CD40 antibody or antigen-binding fragment thereof can be formulated as a liquid pharmaceutical formulation in the manner set forth herein. Where the antagonist anti-CD40 antibody or antigen-binding fragment thereof is to be stored prior to its formulation, it can be frozen, for example, at  $\leq -20^{\circ}\text{C}$ , and then thawed at room temperature for further formulation. The liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. The amount of antibody or antigen-binding fragment thereof present in the formulation takes into consideration the route of administration and desired dose volume.

In this manner, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, about 0.5 mg/ml to about 40.0 mg/ml, about 1.0 mg/ml to about 30.0 mg/ml, about 5.0 mg/ml to about 25.0 mg/ml, about 5.0 mg/ml to about 20.0 mg/ml, or about 15.0 mg/ml to about 25.0 mg/ml. In some embodiments, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody or antigen-binding fragment thereof at a

concentration of about 0.1 mg/ml to about 5.0 mg/ml, about 5.0 mg/ml to about 10.0 mg/ml, about 10.0 mg/ml to about 15.0 mg/ml, about 15.0 mg/ml to about 20.0 mg/ml, about 20.0 mg/ml to about 25.0 mg/ml, about 25.0 mg/ml to about 30.0 mg/ml, about 30.0 mg/ml to about 35.0 mg/ml, about 35.0 mg/ml to about 40.0 mg/ml, about 40.0 mg/ml to about 45.0 mg/ml, or about 45.0 mg/ml to about 50.0 mg/ml. In other  
5       embodiments, the liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody or antigen-binding fragment thereof at a concentration of about 15.0 mg/ml, about 16.0 mg/ml, about 17.0 mg/ml, about 18.0 mg/ml, about 19.0 mg/ml, about 20.0 mg/ml, about 21.0 mg/ml, about 22.0 mg/ml, about 23.0 mg/ml, about 24.0 mg/ml, or  
10       about 25.0 mg/ml. The liquid pharmaceutical composition comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 antibody, or antigen-binding fragment thereof and a buffer that maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, including about pH 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and other such values within  
15       the range of about pH 5.0 to about pH 7.0. In some embodiments, the buffer maintains the pH of the formulation in the range of about pH 5.0 to about pH 6.5, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about 7.0, about pH 5.5 to about pH 6.5, or about pH 5.5 to about pH 6.0.

Any suitable buffer that maintains the pH of the liquid anti-CD40 antibody  
20       formulation in the range of about pH 5.0 to about pH 7.0 can be used in the formulation, so long as the physicochemical stability and desired biological activity of the antibody are retained as noted herein above. Suitable buffers include, but are not limited to, conventional acids and salts thereof, where the counter ion can be, for example, sodium, potassium, ammonium, calcium, or magnesium. Examples of conventional acids and  
25       salts thereof that can be used to buffer the pharmaceutical liquid formulation include, but are not limited to, succinic acid or succinate, citric acid or citrate, acetic acid or acetate, tartaric acid or tartarate, phosphoric acid or phosphate, gluconic acid or gluconate, glutamic acid or glutamate, aspartic acid or aspartate, maleic acid or maleate, and malic acid or malate buffers. The buffer concentration within the formulation can be from  
30       about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values

within the range of about 1 mM to about 50 mM. In some embodiments, the buffer concentration within the formulation is from about 5 mM to about 15 mM, including about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, or other such values within the range of about 5 mM to about 15 mM.

5 In some embodiments of the invention, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof and succinate buffer or citrate buffer at a concentration that maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, preferably about  
10 pH 5.0 to about pH 6.5. By "succinate buffer" or "citrate buffer" is intended a buffer comprising a salt of succinic acid or a salt of citric acid, respectively. In a preferred embodiment, the succinate or citrate counterion is the sodium cation, and thus the buffer is sodium succinate or sodium citrate, respectively. However, any cation is expected to be effective. Other possible succinate or citrate cations include, but are not limited to,  
15 potassium, ammonium, calcium, and magnesium. As noted above, the succinate or citrate buffer concentration within the formulation can be from about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values within the range of about 1 mM to about 50 mM. In some embodiments, the buffer concentration within the  
20 formulation is from about 5 mM to about 15 mM, including about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or about 15 mM. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, or  
25 about 5.0 mg/ml to about 25.0 mg/ml, and succinate or citrate buffer, for example, sodium succinate or sodium citrate buffer, at a concentration of about 1 mM to about 20 mM, about 5 mM to about 15 mM, preferably about 10 mM.

Where it is desirable for the liquid pharmaceutical formulation to be near isotonic, the liquid pharmaceutical formulation comprising a therapeutically effective amount of  
30 the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the

pH of the formulation within the range of about pH 5.0 to about pH 7.0 can further comprise an amount of an isotonicizing agent sufficient to render the formulation near isotonic. By "near isotonic" is intended the aqueous formulation has an osmolarity of about 240 mmol/kg to about 360 mmol/kg, preferably about 240 to about 340 mmol/kg, more preferably about 250 to about 330 mmol/kg, even more preferably about 260 to about 320 mmol/kg, still more preferably about 270 to about 310 mmol/kg. Methods of determining the isotonicity of a solution are known to those skilled in the art. See, for example, Setnikar *et al.* (1959) *J. Am. Pharm. Assoc.* 48:628.

Those skilled in the art are familiar with a variety of pharmaceutically acceptable solutes useful in providing isotonicity in pharmaceutical compositions. The isotonicizing agent can be any reagent capable of adjusting the osmotic pressure of the liquid pharmaceutical formulation of the present invention to a value nearly equal to that of a body fluid. It is desirable to use a physiologically acceptable isotonicizing agent. Thus, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, can further comprise components that can be used to provide isotonicity, for example, sodium chloride; amino acids such as alanine, valine, and glycine; sugars and sugar alcohols (polyols), including, but not limited to, glucose, dextrose, fructose, sucrose, maltose, mannitol, trehalose, glycerol, sorbitol, and xylitol; acetic acid, other organic acids or their salts, and relatively minor amounts of citrates or phosphates. The ordinary skilled person would know of additional agents that are suitable for providing optimal tonicity of the liquid formulation.

In some preferred embodiments, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, further comprises sodium chloride as the isotonicizing agent. The concentration of sodium chloride in the formulation will depend upon the contribution of other components to tonicity. In some embodiments, the concentration of sodium chloride is about 50 mM to about 300 mM, about 50 mM to about 250 mM, about

50 mM to about 200 mM, about 50 mM to about 175 mM, about 50 mM to about 150 mM, about 75 mM to about 175 mM, about 75 mM to about 150 mM, about 100 mM to about 175 mM, about 100 mM to about 200 mM, about 100 mM to about 150 mM, about 125 mM to about 175 mM, about 125 mM to about 150 mM, about 130 mM to about 170 mM, about 130 mM to about 160 mM, about 135 mM to about 155 mM, about 140 mM to about 155 mM, or about 145 mM to about 155 mM. In one such embodiment, the concentration of sodium chloride is about 150 mM. In other such embodiments, the concentration of sodium chloride is about 150 mM, the buffer is sodium succinate or sodium citrate buffer at a concentration of about 5 mM to about 15 mM, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, and the formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, at a concentration of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, about 150 mM sodium chloride, and about 10 mM sodium succinate or sodium citrate, at a pH of about pH 5.5.

Protein degradation due to freeze thawing or mechanical shearing during processing of a liquid pharmaceutical formulations of the present invention can be inhibited by incorporation of surfactants into the formulation in order to lower the surface tension at the solution-air interface. Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH 7.0, and further comprises a surfactant. In other embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, a buffer to maintain the pH of the formulation within the range of about pH 5.0 to about pH

7.0, an isotonizing agent such as sodium chloride at a concentration of about 50 mM to about 300 mM, and further comprises a surfactant.

Typical surfactants employed are nonionic surfactants, including polyoxyethylene sorbitol esters such as polysorbate 80 (Tween 80) and polysorbate 20 (Tween 20);

polyoxypropylene-polyoxyethylene esters such as Pluronic F68; polyoxyethylene alcohols such as Brij 35; simethicone; polyethylene glycol such as PEG400; lysophosphatidylcholine; and polyoxyethylene-p-t-octylphenol such as Triton X-100.

Classic stabilization of pharmaceuticals by surfactants or emulsifiers is described, for example, in Levine *et al.* (1991) *J. Parenteral Sci. Technol.* 45(3):160-165, herein

incorporated by reference. A preferred surfactant employed in the practice of the present invention is polysorbate 80. Where a surfactant is included, it is typically added in an amount from about 0.001 % to about 1.0% (w/v), about 0.001% to about 0.5%, about 0.001% to about 0.4%, about 0.001% to about 0.3%, about 0.001% to about 0.2%, about 0.005% to about 0.5%, about 0.005% to about 0.2%, about 0.01% to about 0.5%, about 0.01% to about 0.2%, about 0.03% to about 0.5%, about 0.03% to about 0.3%, about 0.05% to about 0.5%, or about 0.05% to about 0.2%.

Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, the

buffer is sodium succinate or sodium citrate buffer at a concentration of about 1 mM to about 50 mM, about 5 mM to about 25 mM, or about 5 mM to about 15 mM; the formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5; and the formulation further comprises a surfactant, for example, polysorbate 80, in an amount from about 0.001% to about 1.0% or about

0.001% to about 0.5%. Such formulations can optionally comprise an isotonizing agent, such as sodium chloride at a concentration of about 50 mM to about 300 mM, about 50 mM to about 200 mM, or about 50 mM to about 150 mM. In other embodiments, the liquid pharmaceutical formulation comprises the antagonist anti-CD40 antibody, for example, the CHIR-12.12 or CHIR-5.9 monoclonal antibody, or antigen-binding fragment thereof, at a concentration of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, including about 20.0 mg/ml; about 50 mM to about 200 mM

sodium chloride, including about 150 mM sodium chloride; sodium succinate or sodium citrate at about 5 mM to about 20 mM, including about 10 mM sodium succinate or sodium citrate; sodium chloride at a concentration of about 50 mM to about 200 mM, including about 150 mM; and optionally a surfactant, for example, polysorbate 80, in an amount from about 0.001% to about 1.0%, including about 0.001% to about 0.5%; where the liquid pharmaceutical formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about pH 6.5, or about pH 5.5 to about pH 6.0.

The liquid pharmaceutical formulation can be essentially free of any preservatives and other carriers, excipients, or stabilizers noted herein above. Alternatively, the formulation can include one or more preservatives, for example, antibacterial agents, pharmaceutically acceptable carriers, excipients, or stabilizers described herein above provided they do not adversely affect the physicochemical stability of the antagonist anti-CD40 antibody or antigen-binding fragment thereof. Examples of acceptable carriers, excipients, and stabilizers include, but are not limited to, additional buffering agents, co-solvents, surfactants, antioxidants including ascorbic acid and methionine, chelating agents such as EDTA, metal complexes (for example, Zn-protein complexes), and biodegradable polymers such as polyesters. A thorough discussion of formulation and selection of pharmaceutically acceptable carriers, stabilizers, and isomolytes can be found in *Remington's Pharmaceutical Sciences* (18<sup>th</sup> ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference.

After the liquid pharmaceutical formulation or other pharmaceutical composition described herein is prepared, it can be lyophilized to prevent degradation. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) that may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

### Use of Antagonist Anti-CD40 Antibodies in the Manufacture of Medicaments



The present invention also provides for the use of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a subject for a cancer characterized by neoplastic B cell growth, wherein the medicament is coordinated with treatment using an anti-CD20 antibody or antigen-binding fragment thereof. Such cancers include, but are not limited to, the B cell-related cancers discussed herein above, for example, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia, myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma, follicular small cleaved lymphoma, follicular large cell lymphoma, follicular mixed small cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma, mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.

By "coordinated" is intended the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof is to be used either prior to, during, or after treatment of the subject using an anti-CD20 antibody or antigen-binding fragment thereof. In one such embodiment, the present invention provides for the use of the monoclonal antibody CHIR-12.12 or CHIR-5.9 in the manufacture of a medicament for treating a B cell-related cancer in a subject, wherein the medicament is coordinated with treatment using an anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen-binding fragment thereof, wherein the medicament is to be used either prior to, during, or after treatment of the subject using the anti-CD20 antibody or antigen-binding fragment thereof.

In some embodiments, the medicament comprising the antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof is coordinated with treatment using an anti-

CD20 antibody or antigen-binding fragment thereof and at least one other type of cancer therapy. Examples of other cancer therapies include, but are not limited to, those described herein above, i.e., surgery; radiation therapy; chemotherapy, optionally in combination with autologous bone marrow transplant, where suitable chemotherapeutic agents include, where suitable chemotherapeutic agents include, but are not limited to, fludarabine or fludarabine phosphate, chlorambucil, vincristine, pentostatin, 2-chlorodeoxyadenosine (cladribine), cyclophosphamide, doxorubicin, prednisone, and combinations thereof, for example, anthracycline-containing regimens such as CAP (cyclophosphamide, doxorubicin plus prednisone), CHOP (cyclophosphamide, vincristine, prednisone plus doxorubicin), VAD (vincristine, doxorubicin, plus dexamethasone), MP (melphalan plus prednisone), and other cytotoxic and/or therapeutic agents used in chemotherapy such as mitoxantrone, daunorubicin, idarubicin, asparaginase, and antimetabolites, including, but not limited to, cytarabine, methotrexate, 5-fluorouracil decarbazine, 6-thioguanine, 6-mercaptopurine, and nelarabine; other anti-cancer monoclonal antibody therapy (for example, alemtuzumab (Campath<sup>®</sup>) or other anti-CD52 antibody targeting the CD52 cell-surface glycoprotein on malignant B cells; anti-CD19 antibody (for example, MT103, a bispecific antibody); anti-CD22 antibody (for example, the humanized monoclonal antibody epratuzumab); bevacizumab (Avastin<sup>®</sup>) or other anti-cancer antibody targeting human vascular endothelial growth factor; anti-CD22 antibody targeting the CD22 antigen on malignant B cells (for example, the monoclonal antibody BL-22, an alphaCD22 toxin);  $\alpha$ -M-CSF antibody targeting macrophage colony stimulating factor; antibodies targeting the receptor activator of nuclear factor-kappaB (RANK) and its ligand (RANKL), which are overexpressed in multiple myeloma; anti-CD23 antibody targeting the CD23 antigen on malignant B cells (for example, IDEC-152); anti-CD80 antibody targeting the CD80 antigen (for example, IDEC-114); anti-CD38 antibody targeting the CD38 antigen on malignant B cells; antibodies targeting major histocompatibility complex class II receptors (anti-MHC antibodies) expressed on malignant B cells; other anti-CD40 antibodies (for example, SGN-40) targeting the CD40 antigen on malignant B cells; and antibodies targeting tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) (for example, the agonistic human monoclonal antibody HGS-ETR1) and

TRAIL-R2 expressed on a number of solid tumors and tumors of hematopoietic origin); small molecule-based cancer therapy, including, but not limited to, microtubule and/or topoisomerase inhibitors (for example, the mitotic inhibitor dolastatin and dolastatin analogues; the tubulin-binding agent T900607; XL119; and the topoisomerase inhibitor aminocamptothecin), SDX-105 (bendamustine hydrochloride), ixabepilone (an epothilone analog, also referred to as BMS-247550), protein kinase C inhibitors, for example, midostaurin ((PKC-412, CGP 41251, N-benzoylstaurosporine), pixantrone, eloxatin (an antineoplastic agent), ganite (gallium nitrate), Thalomid<sup>®</sup> (thalidomide), immunomodulatory derivatives of thalidomide (for example, revlimid (formerly revimid)), Affinitak<sup>™</sup> (antisense inhibitor of protein kinase C-alpha), SDX-101 (R-etodolac, inducing apoptosis of malignant lymphocytes), second-generation purine nucleoside analogs such as clofarabine, inhibitors of production of the protein Bcl-2 by cancer cells (for example, the antisense agents oblimersen and Genasense<sup>®</sup>), proteasome inhibitors (for example, Velcade<sup>™</sup> (bortezomib)), small molecule kinase inhibitors (for example, CHIR-258), small molecule VEGF inhibitors (for example, ZD-6474), small molecule inhibitors of heat shock protein (HSP) 90 (for example, 17-AAG), small molecule inhibitors of histone deacetylases (for example, hybrid/polar cytodifferentiation HPC) agents such as suberanilohydroxamic acid (SAHA), and FR-901228) and apoptotic agents such as Trisenox<sup>®</sup> (arsenic trioxide) and Xcytrin<sup>®</sup> (motexafin gadolinium); vaccine /immunotherapy-based cancer therapies, including, but not limited to, vaccine approaches (for example, Id-KLH, oncopophage, vitalethine), personalized immunotherapy or active idiotype immunotherapy (for example, MyVax<sup>®</sup> Personalized Immunotherapy, formally designated GTOP-99), Promune<sup>®</sup> (CpG 7909, a synthetic agonist for toll-like receptor 9 (TLR9)), interferon-alpha therapy, interleukin-2 (IL-2) therapy, IL-12 therapy, IL-15 therapy, and IL-21 therapy; steroid therapy; or other cancer therapy; where treatment with the anti-CD20 antibody or antigen-binding fragment thereof and the additional cancer therapy, or additional cancer therapies, occurs prior to, during, or subsequent to treatment of the subject with the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof, as noted herein above. Where the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof is coordinated with treatment using an anti-CD20 antibody or antigen-

binding fragment thereof and at least one other cancer therapy, use of the medicament can be prior to, during, or after treatment of the subject with either or both of the other cancer therapies.

The present invention also provides for the use of a synergistic combination of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a subject for a cancer characterized by neoplastic B cell growth, including the B cell-related cancers described herein above, wherein the medicament is coordinated with treatment using an anti-CD20 antibody or antigen-binding fragment thereof. By “synergistic combination” is intended the medicament comprises an amount of the antagonist anti-CD40 antibody or antigen-binding fragment thereof that provides for a synergistic therapeutic effect when the medicament is coordinated with treatment using an anti-CD20 antibody or antigen-binding fragment thereof in the manner set forth herein above. “Synergistic therapeutic effect” refers to a therapeutic effect observed with a combination of two or more therapies (in this case, the antagonist anti-CD40 antibody therapy and anti-CD20 antibody therapy) wherein the therapeutic effect (as measured by any of a number of parameters, including the measures of efficacy described herein above) is greater than the sum of the respective individual therapeutic effects observed with the respective individual therapies.

In one such embodiment, the present invention provides for the use of a synergistic combination of the monoclonal antibody CHIR-12.12 or CHIR-5.9 in the manufacture of a medicament for treating a B cell-related cancer in a subject, wherein the medicament is coordinated with treatment using an anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen-binding fragment thereof, wherein the medicament is to be used either prior to, during, or after treatment of the subject using the anti-CD20 antibody or antigen-binding fragment thereof. In some embodiments, the medicament comprising the synergistic combination of the antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof is coordinated with treatment using an anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen binding fragment thereof and at least one other type of cancer therapy as noted herein above.

The invention also provides for the use of an antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof in the manufacture of a medicament for treating a subject for a cancer characterized by neoplastic B cell growth, including the B cell-related cancers described herein above, wherein the medicament is used in a subject that has been pretreated with an anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen-binding fragment thereof. By “pretreated” or “pretreatment” is intended the subject has received anti-CD20 antibody therapy (i.e., been treated using an anti-CD20 antibody or antigen-binding fragment thereof) prior to receiving the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof. “Pretreated” or “pretreatment” includes subjects that have been treated using an anti-CD20 antibody or antigen-binding fragment thereof variant thereof, alone or in combination with other cancer therapies, within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or even within 1 day prior to initiation of treatment with the medicament comprising the antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof. It is not necessary that the subject was a responder to pretreatment with the prior anti-CD20 antibody therapy, or prior anti-CD20 antibody therapy and other cancer therapies. Thus, the subject that receives the medicament comprising the antagonist anti-CD40 antibody or antigen-binding fragment thereof could have responded, or could have failed to respond (i.e. the cancer was refractory), to pretreatment with the prior anti-CD20 antibody therapy, or to one or more of the prior cancer therapies where pretreatment comprised multiple cancer therapies one of which was anti-CD20 antibody therapy, for example, anti-CD20 antibody therapy and surgery; anti-CD20 antibody therapy and chemotherapy; anti-CD20 antibody therapy and IL-2 therapy; or anti-CD20 antibody therapy, chemotherapy, and IL-2 therapy.

Thus, in some embodiments, the invention provides for the use of an antagonist anti-CD40 antibody, for example the monoclonal antibody CHIR-12.12 or CHIR-5.9 disclosed herein, or antigen-binding fragment thereof in the manufacture of a medicament

that is to be used in a subject in need of treatment for a cancer characterized by neoplastic B cell growth, for example, a B cell-related cancer such as that described herein above, where the subject has been pretreated with anti-CD20 antibody therapy, or has been pretreated with anti-CD20 antibody therapy and one or more of the following other

5 cancer therapies: surgery; radiation therapy; chemotherapy, optionally in combination with autologous bone marrow transplant, where suitable chemotherapeutic agents include, but are not limited to, fludarabine or fludarabine phosphate, chlorambucil, vincristine, pentostatin, 2-chlorodeoxyadenosine (cladribine), cyclophosphamide, doxorubicin, prednisone, and combinations thereof, for example, anthracycline-

10 containing regimens such as CAP (cyclophosphamide, doxorubicin plus prednisone), CHOP (cyclophosphamide, vincristine, prednisone plus doxorubicin), VAD (vincristine, doxorubicin, plus dexamethasone), MP (melphalan plus prednisone), and other cytotoxic and/or therapeutic agents used in chemotherapy such as mitoxantrone, daunorubicin, idarubicin, asparaginase, and antimetabolites, including, but not limited to, cytarabine,

15 methotrexate, 5-fluorouracil decarbazine, 6-thioguanine, 6-mercaptopurine, and nelarabine; other anti-cancer monoclonal antibody therapy (for example, alemtuzumab (Campath<sup>®</sup>) or other anti-CD52 antibody targeting the CD52 cell-surface glycoprotein on malignant B cells; anti-CD19 antibody (for example, MT103, a bispecific antibody); anti-CD22 antibody (for example, the humanized monoclonal antibody epratuzumab);

20 bevacizumab (Avastin<sup>®</sup>) or other anti-cancer antibody targeting human vascular endothelial growth factor; anti-CD22 antibody targeting the CD22 antigen on malignant B cells (for example, the monoclonal antibody BL-22, an alphaCD22 toxin);  $\alpha$ -M-CSF antibody targeting macrophage colony stimulating factor; antibodies targeting the receptor activator of nuclear factor-kappaB (RANK) and its ligand (RANKL), which are

25 overexpressed in multiple myeloma; anti-CD23 antibody targeting the CD23 antigen on malignant B cells (for example, IDEC-152); anti-CD80 antibody targeting the CD80 antigen (for example, IDEC-114); anti-CD38 antibody targeting the CD38 antigen on malignant B cells; antibodies targeting major histocompatibility complex class II receptors (anti-MHC antibodies) expressed on malignant B cells; other anti-CD40

30 antibodies (for example, SGN-40) targeting the CD40 antigen on malignant B cells; and antibodies targeting tumor necrosis factor-related apoptosis-inducing ligand receptor 1

(TRAIL-R1) (for example, the agonistic human monoclonal antibody HGS-ETR1) and TRAIL-R2 expressed on a number of solid tumors and tumors of hematopoietic origin); small molecule-based cancer therapy, including, but not limited to, microtubule and/or topoisomerase inhibitors (for example, the mitotic inhibitor dolastatin and dolastatin analogues; the tubulin-binding agent T900607; XL119; and the topoisomerase inhibitor aminocamptothecin), SDX-105 (bendamustine hydrochloride), ixabepilone (an epothilone analog, also referred to as BMS-247550), protein kinase C inhibitors, for example, midostaurin ((PKC-412, CGP 41251, N-benzoylstauroporine), pixantrone, eloxatin (an antineoplastic agent), ganite (gallium nitrate), Thalomid<sup>®</sup> (thalidomide), immunomodulatory derivatives of thalidomide (for example, revlimid (formerly revimid)), Affinitak<sup>™</sup> (antisense inhibitor of protein kinase C- $\alpha$ ), SDX-101 (R-etodolac, inducing apoptosis of malignant lymphocytes), second-generation purine nucleoside analogs such as clofarabine, inhibitors of production of the protein Bcl-2 by cancer cells (for example, the antisense agents oblimersen and Genasense<sup>®</sup>), proteasome inhibitors (for example, Velcade<sup>™</sup> (bortezomib)), small molecule kinase inhibitors (for example, CHIR-258), small molecule VEGF inhibitors (for example, ZD-6474), small molecule inhibitors of heat shock protein (HSP) 90 (for example, 17-AAG), small molecule inhibitors of histone deacetylases (for example, hybrid/polar cytodifferentiation HPC) agents such as suberanilohydroxamic acid (SAHA), and FR-901228) and apoptotic agents such as Trisenox<sup>®</sup> (arsenic trioxide) and Xcytrin<sup>®</sup> (motexafin gadolinium); vaccine /immunotherapy-based cancer therapies, including, but not limited to, vaccine approaches (for example, Id-KLH, oncoplage, vitaethine), personalized immunotherapy or active idiotype immunotherapy (for example, MyVax<sup>®</sup> Personalized Immunotherapy, formally designated GTO-99), Promune<sup>®</sup> (CpG 7909, a synthetic agonist for toll-like receptor 9 (TLR9)), interferon- $\alpha$  therapy, interleukin-2 (IL-2) therapy, IL-12 therapy, IL-15 therapy, and IL-21 therapy; steroid therapy; or other cancer therapy.

The present invention also provides for the use of an anti-CD20 antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a subject for a cancer characterized by neoplastic B cell growth, including a B cell-related cancer, wherein the medicament is coordinated with treatment using an antagonist anti-CD40 antibody or antigen binding fragment thereof. In these embodiments,

“coordinated” is intended to mean the medicament comprising the anti-CD20 antibody or antigen-binding fragment thereof is to be used either prior to, during, or after treatment of the subject using the antagonist anti-CD40 antibody or antigen-binding fragment thereof.

In one such embodiment, the present invention provides for the use of an anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen-binding fragment thereof in the manufacture of a medicament for treating a subject for a cancer characterized by neoplastic B cell growth, such as a B cell-related cancer, wherein the medicament is coordinated with treatment using the monoclonal antibody CHIR-12.12 or CHIR-5.9, wherein the medicament is to be used either prior to, during, or after treatment of the subject with the monoclonal antibody CHIR-12.12 or CHIR-5.9.

In some embodiments, the medicament comprising the anti-CD20 antibody, for example, rituximab (Rituxan®), or antigen-binding fragment thereof is coordinated with treatment using an antagonist anti-CD40 antibody, for example, the monoclonal antibody CHIR-12.12 or CHIR-5.9, or antigen-binding fragment thereof, and at least one other type of cancer therapy. Examples of other cancer therapies include, but are not limited to, those described herein above, i.e., surgery; radiation therapy; chemotherapy, optionally in combination with autologous bone marrow transplant, where suitable chemotherapeutic agents include, but are not limited to, fludarabine or fludarabine phosphate, chlorambucil, vincristine, pentostatin, 2-chlorodeoxyadenosine (cladribine), cyclophosphamide, doxorubicin, prednisone, and combinations thereof, for example, anthracycline-containing regimens such as CAP (cyclophosphamide, doxorubicin plus prednisone), CHOP (cyclophosphamide, vincristine, prednisone plus doxorubicin), VAD (vincristine, doxorubicin, plus dexamethasone), MP (melphalan plus prednisone), and other cytotoxic and/or therapeutic agents used in chemotherapy such as mitoxantrone, daunorubicin, idarubicin, asparaginase, and antimetabolites, including, but not limited to, cytarabine, methotrexate, 5-fluorouracil decarbazine, 6-thioguanine, 6-mercaptopurine, and nelarabine; other anti-cancer monoclonal antibody therapy (for example, alemtuzumab (Campath®) or other anti-CD52 antibody targeting the CD52 cell-surface glycoprotein on malignant B cells; anti-CD19 antibody (for example, MT103, a bispecific antibody); anti-CD22 antibody (for example, the humanized monoclonal antibody epratuzumab); bevacizumab (Avastin®) or other anti-cancer antibody targeting



human vascular endothelial growth factor; anti-CD22 antibody targeting the CD22 antigen on malignant B cells (for example, the monoclonal antibody BL-22, an alphaCD22 toxin);  $\alpha$ -M-CSF antibody targeting macrophage colony stimulating factor; antibodies targeting the receptor activator of nuclear factor-kappaB (RANK) and its ligand (RANKL), which are overexpressed in multiple myeloma; anti-CD23 antibody targeting the CD23 antigen on malignant B cells (for example, IDEC-152); anti-CD80 antibody targeting the CD80 antigen (for example, IDEC-114); anti-CD38 antibody targeting the CD38 antigen on malignant B cells; antibodies targeting major histocompatibility complex class II receptors (anti-MHC antibodies) expressed on malignant B cells; other anti-CD40 antibodies (for example, SGN-40) targeting the CD40 antigen on malignant B cells; and antibodies targeting tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) (for example, the agonistic human monoclonal antibody HGS-ETR1) and TRAIL-R2 expressed on a number of solid tumors and tumors of hematopoietic origin); small molecule-based cancer therapy, including, but not limited to, microtubule and/or topoisomerase inhibitors (for example, the mitotic inhibitor dolastatin and dolastatin analogues; the tubulin-binding agent T900607; XL119; and the topoisomerase inhibitor aminocamptothecin), SDX-105 (bendamustine hydrochloride), ixabepilone (an epothilone analog, also referred to as BMS-247550), protein kinase C inhibitors, for example, midostaurin ((PKC-412, CGP 41251, N-benzoylstauroporine), pixantrone, eloxatin (an antineoplastic agent), ganite (gallium nitrate), Thalomid<sup>®</sup> (thalidomide), immunomodulatory derivatives of thalidomide (for example, revlimid (formerly revimid)), Affinitak<sup>™</sup> (antisense inhibitor of protein kinase C-alpha), SDX-101 (R-etodolac, inducing apoptosis of malignant lymphocytes), second-generation purine nucleoside analogs such as clofarabine, inhibitors of production of the protein Bcl-2 by cancer cells (for example, the antisense agents oblimersen and Genasense<sup>®</sup>), proteasome inhibitors (for example, Velcade<sup>™</sup> (bortezomib)), small molecule kinase inhibitors (for example, CHIR-258), small molecule VEGF inhibitors (for example, ZD-6474), small molecule inhibitors of heat shock protein (HSP) 90 (for example, 17-AAG), small molecule inhibitors of histone deacetylases (for example, hybrid/polar cytodifferentiation HPC) agents such as suberanilohydroxamic acid (SAHA), and FR-901228) and apoptotic agents such as Trisenox<sup>®</sup> (arsenic trioxide) and

Xcytrin® (motexafin gadolinium); vaccine /immunotherapy-based cancer therapies, including, but not limited to, vaccine approaches (for example, Id-KLH, oncopage, vitaethine), personalized immunotherapy or active idiotype immunotherapy (for example, MyVax® Personalized Immunotherapy, formally designated GTOP-99),  
5 Promune® (CpG 7909, a synthetic agonist for toll-like receptor 9 (TLR9)), interferon-alpha therapy, interleukin-2 (IL-2) therapy, IL-12 therapy, IL-15 therapy, and IL-21 therapy; steroid therapy; or other cancer therapy; where treatment with the antagonist anti-CD40 antibody or antigen-binding fragment thereof and the additional cancer therapy, or additional cancer therapies, occurs prior to, during, or subsequent to treatment  
10 of the subject with the medicament comprising the anti-CD20 antibody or antigen-binding fragment thereof. Where the medicament comprising the anti-CD20 antibody or antigen-binding fragment thereof is coordinated with treatment using the antagonist anti-CD40 antibody or antigen-binding fragment thereof and at least one other cancer therapy, use of the medicament can be prior to, during, or after treatment of the subject with either  
15 or both of the other cancer therapies.

“Treatment” in the context of coordinated use of a medicament described herein with one or more other cancer therapies is herein defined as the application or administration of the medicament or of the other cancer therapy to a subject, or application or administration of the medicament or other cancer therapy to an isolated  
20 tissue or cell line from a subject, where the subject has a cancer characterized by neoplastic B cell growth, a symptom associated with such a cancer, or a predisposition toward development of such a cancer, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the cancer, any associated symptoms of the cancer, or the predisposition toward the development of the cancer.

25 The following examples are offered by way of illustration and not by way of limitation.

## EXPERIMENTAL

### *Introduction*

30 The antagonist anti-CD40 antibodies used in the examples below are CHIR-5.9 and CHIR-12.12. The CHIR-5.9 and CHIR-12.12 anti-CD40 antibodies are human IgG<sub>1</sub>

subtype anti-human CD40 monoclonal antibodies (mAbs) generated by immunization of transgenic mice bearing the human IgG<sub>1</sub> heavy chain locus and the human  $\kappa$  light chain locus (XenoMouse<sup>®</sup> technology (Abgenix; Fremont, California)). SF9 insect cells expressing CD40 extracellular domain were used as immunogen.

5 Briefly, splenocytes from immunized mice were fused with SP 2/0 or P 3 x 63Ag8.653 murine myeloma cells at a ratio of 10:1 using 50% polyethylene glycol as previously described by de Boer *et al.* (1988) *J. Immunol. Meth.* 113:143. The fused cells were resuspended in complete IMDM medium supplemented with hypoxanthine ( 0.1 mM), aminopterin ( 0.01 mM), thymidine ( 0.016 mM), and 0.5 ng/ml hIL-6 (Genzyme,  
10 Cambridge, Massachusetts). The fused cells were then distributed between the wells of 96-well tissue culture plates, so that each well contained 1 growing hybridoma on average.

After 10-14 days, the supernatants of the hybridoma populations were screened for specific antibody production. For the screening of specific antibody production by  
15 the hybridoma clones, the supernatants from each well were pooled and tested for anti-CD40 activity specificity by ELISA first. The positives were then used for fluorescent cell staining of EBV-transformed B cells using a standard FACS assay. Positive hybridoma cells were cloned twice by limiting dilution in IMDM/FBS containing 0.5 ng/ml hIL-6.

20 A total of 31 mice spleens were fused with the mouse myeloma SP2/0 cells to generate 895 antibodies that recognize recombinant CD40 in ELISA. On average approximately 10% of hybridomas produced using Abgenix XenoMouse<sup>®</sup> technology (Abgenix; Fremont, California) may contain mouse lambda light chain instead of human kappa chain. The antibodies containing mouse light lambda chain were selected out. A  
25 subset of 260 antibodies that also showed binding to cell-surface CD40 were selected for further analysis. Stable hybridomas selected during a series of subcloning procedures were used for further characterization in binding and functional assays. For further details of the selection process, see copending provisional applications entitled  
"Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use," filed  
30 November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)),

60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety.

Clones from 7 other hybridomas were identified as having antagonistic activity.

- 5 Based on their relative antagonistic potency and ADCC activities, two hybridoma clones were selected for further evaluation (Table 1 below). They are named 131.2F8.5.9 (5.9) and 153.8E2.D10.D6.12.12 (12.12).

Table 1. Summary of initial set of data with anti-CD40 IgG1 antibodies CHIR-5.9 and CHIR-12.12.

| Mother Hybridoma | Hybridoma clones   | Cell surface binding | Antagonist | ADCC | CDC | CMCC# | V-region DNA sequence |
|------------------|--------------------|----------------------|------------|------|-----|-------|-----------------------|
| 131.2F5          | 131.2F5.8.5.9      | +++                  | +++        | ++   | -   | 12047 | Yes                   |
| 153.8E2          | 153.8E2D10D6.12.12 | +++                  | +++        | ++++ | -   | 12056 | Yes                   |

10 Mouse hybridoma line 131.2F8.5.9 (CMCC#12047) and hybridoma line 153.8E2.D10.D6.12.12 (CMCC#12056) have been deposited with the American Type Culture Collection (ATCC; 10801 University Blvd., Manassas, Virginia 20110-2209 (USA)) under Patent Deposit Number PTA-5542 and PTA-5543, respectively.

- 15 The cDNAs encoding the variable regions of the candidate antibodies were amplified by PCR, cloned, and sequenced. The amino acid sequences for the light chain and heavy chain of the CHIR-12.12 antibody are set forth in Figures 2A and 2B, respectively. See also SEQ ID NO:2 (light chain for mAb CHIR-12.12) and SEQ ID NO:4 (heavy chain for mAb CHIR-12.12). A variant of the heavy chain for mAb CHIR-12.12 is shown in Figure 2B (see also SEQ ID NO:5), which differs from SEQ ID NO:4 in having a serine residue substituted for the alanine residue at position 153 of SEQ ID NO:4. The nucleotide sequences encoding the light chain and heavy chain of the CHIR-12.12 antibody are set forth in Figures 3A and 3B, respectively. See also SEQ ID NO:1 (coding sequence for light chain for mAb CHIR-12.12) and SEQ ID NO:3 (coding sequence for heavy chain for mAb CHIR-12.12). The amino acid sequences for the light chain and heavy chain of the CHIR-5.9 antibody are set forth in Figures 4A and 4B, respectively. See also SEQ ID NO:6 (light chain for mAb CHIR-5.9) and SEQ ID NO:7 (heavy chain for mAb CHIR-5.9). A variant of the heavy chain for mAb CHIR-5.9 is
- 20
- 25

shown in Figure 3B (see also SEQ ID NO:8), which differs from SEQ ID NO:7 in having a serine residue substituted for the alanine residue at position 158 of SEQ ID NO:7.

As expected for antibodies derived from independent hybridomas, there is substantial variation in the nucleotide sequences in the complementarity determining regions (CDRs). The diversity in the CDR3 region of V<sub>H</sub> is believed to most significantly determine antibody specificity.

As shown by FACS analysis, CHIR-5.9 and CHIR-12.12 bind specifically to human CD40 and can prevent CD40-ligand binding. Both mAbs can compete off CD40-ligand pre-bound to cell surface CD40. The binding affinity of CHIR-5.9 to human CD40 is  $1.2 \times 10^{-8}$  M and the binding affinity of CHIR-12.12 to human CD40 is  $5 \times 10^{-10}$  M.

The CHIR-12.12 and CHIR-5.9 monoclonal antibodies are strong antagonists and inhibit *in vitro* CD40 ligand-mediated proliferation of normal B cells, as well as inhibiting *in vitro* CD40 ligand-mediated proliferation of cancer cells from NHL and CLL patients. *In vitro*, both antibodies kill primary cancer cells from NHL patients by ADCC. Dose-dependent anti-tumor activity was seen in a xenograft human lymphoma model. For a more detailed description of these results, and the assays used to obtain them, see copending provisional applications entitled "*Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use*," filed November 4, 2003, November 26, 2003, and April 27, 2004, and assigned U.S. Patent Application Nos. 60/517,337 (Attorney Docket No. PP20107.001 (035784/258442)), 60/525,579 (Attorney Docket No. PP20107.002 (035784/271525)), and 60/565,710 (Attorney Docket No. PP20107.003 (035784/277214)), respectively, the contents of each of which are herein incorporated by reference in their entirety.

Example 1: The Combination of mAb CHIR-12.12 and Rituxan<sup>®</sup> Show Anti-Tumor Activity Against Aggressive, Rituxan<sup>®</sup>-Resistant Burkitt's Lymphoma in a Xenograft Model

Combinations of the chimeric anti-CD20 monoclonal antibody rituximab (Rituxan<sup>®</sup>; IDEC-C2B8; IDEC Pharmaceuticals Corp., San Diego, California) and antagonistic anti-CD40 monoclonal antibody CHIR-12.12 were tested in a murine model.

Specifically, 120 nu/nu, 5-week-old female mice (Charles River Laboratories, Wilmington, MA) underwent an acclimation period of at least 7 days. One day prior to tumor cell inoculation, mice received 3 Gy irradiation using Gammacell 40 Cesium 137 irradiation unit manufactured by Atomic Energy of Canada. Namalwa cells (ATCC, Manassas, VA), a human Rituxan<sup>®</sup>-resistant, aggressive Burkitt's lymphoma cell line, were cultured in RPMI 1640 media with 15% fetal bovine serum. On the day of inoculation, the cells were harvested, counted, and resuspended in 50% HBSS + 50% matrigel at the density of  $5 \times 10^7$  cells/mL. Tumor cells were inoculated subcutaneously at the right flank at  $5 \times 10^6$  cells/100 $\mu$ l/mouse.

One day after tumor inoculation, mice were randomized and injected intraperitoneally (i.p.) once every 7 days (q7d) with anti-CD40 mAb CHIR-12.12 and Rituxan<sup>®</sup> as indicated below:

- a. IgG1, 10 mg/kg, i.p., q7d, x up to 5 doses.
- b. Rituxan<sup>®</sup>, 10 mg/kg, i.p., q7d, x up to 5 doses.
- c. Rituxan<sup>®</sup>, 20 mg/kg, i.p., q7d, x up to 5 doses.
- d. CHIR-12.12, 5 mg/kg, i.p., q7d, x up to 5 doses.
- e. CHIR-12.12, 10 mg/kg, i.p., q7d, x up to 5 doses.
- f. Rituxan<sup>®</sup>, 10 mg/kg + IgG1, 5 mg/kg, i.p., q7d, x up to 5 doses.
- g. Rituxan<sup>®</sup>, 10 mg/kg + CHIR-12.12, 5 mg/kg, i.p., q7d, x up to 5 doses.
- h. Rituxan<sup>®</sup>, 10 mg/kg + IgG1, 10 mg/kg, i.p., q7d, x up to 5 doses.
- i. Rituxan<sup>®</sup>, 10 mg/kg + CHIR-12.12, 10 mg/kg, i.p., q7d, x up to 5 doses.

Tumor volume was measured twice a week using an electronic caliper. When the mean of tumor volume in one group reached 2000 mm<sup>3</sup>, mice in that group were sacrificed. If tumor in treatment group responded, mice were kept until the mean tumor volume reached 2000 mm<sup>3</sup>.

ANOVA was used to analyze the difference of mean tumor volume among all the groups. Tuckey multi-comparison on the Least Squares Means was used to compare the difference of mean tumor volume between two specific groups.

As shown in Figure 1, primary tumor growth was significantly inhibited by i.p. administration of CHIR-12.12 alone at 5 mg/kg once a week for up to 5 weeks (60%, P=0.02). CHIR-12.12 administered alone at 10 mg/kg showed a trend toward significant tumor volume inhibition (39%, P=0.22). Rituxan<sup>®</sup> alone at 10 mg/kg and 20 mg/kg did not inhibit the tumor growth at all. The combination of CHIR-2.12 and Rituxan<sup>®</sup> resulted

in synergistic and CHIR-12.12 dose-dependent tumor growth inhibition with 77% (P=0.001) and 83% (P=0.003) tumor volume inhibition for CHIR-12.12 at 5 mg/kg plus Rituxan<sup>®</sup> at 10 mg/kg and CHIR-12.12 at 10 mg/kg and Rituxan<sup>®</sup> at 10 mg/kg, respectively. No clinical sign of toxicity was observed among all the treated animals under the current doses and regimens. These data suggest that mAb CHIR-12.12 alone is a therapeutic agent for aggressive and Rituxan<sup>®</sup>-resistant lymphoma. However, mAb CHIR-12.12 when used in combination with Rituxan<sup>®</sup> was more efficacious than mAb CHIR-12.12 alone, Rituxan<sup>®</sup> alone, or the sum of the efficacies of these two mAbs used alone.

Example 2: CHIR-5.9 and CHIR-12.12 Bind to a Different Epitope on CD40 than 15B8

The candidate monoclonal antibodies CHIR-5.9 and CHIR-12.12 compete with each other for binding to CD40 but not with 15B8, an IgG<sub>2</sub> anti-CD40 mAb (see International Publication No. WO 02/28904). Antibody competition binding studies using Biacore were designed using CM5 biosensor chips with protein A immobilized via amine coupling, which was used to capture either anti-CD40, CHIR-12.12, or 15B8. Normal association/dissociation binding curves are observed with varying concentrations of CD40-his (data not shown). For competition studies, either CHIR-12.12 or 15B8 were captured onto the protein A surface. Subsequently a CD40-his / CHIR-5.9 Fab complex (100 nM CD40:1  $\mu$ M CHIR-5.9 Fab), at varying concentrations, was flowed across the modified surface. In the case of CHIR-12.12, there was no association of the complex observed, indicating CHIR-5.9 blocks binding of CHIR-12.12 to CD40-his. For 15B8, association of the Fab CHIR-5.9 complex was observed indicating CHIR-5.9 does not block binding of 15B8 to CD40 binding site. However, the off rate of the complex dramatically increased (data not shown).

It has also been determined that 15B8 and CHIR-12.12 do not compete for CD40-his binding. This experiment was set up by capturing CHIR-12.12 on the protein A biosensor chip, blocking residual protein A sites with control hIgG<sub>1</sub>, binding CD40-his and then flowing 15B8 over the modified surface. 15B8 did bind under these conditions indicating CHIR-12.12 does not block 15B8 from binding to CD40.

Example 3: Binding Properties of CHIR-12.12 and CHIR-5.9 mAB

Protein A was immobilized onto CM5 biosensor chips via amine coupling. Human anti-CD40 monoclonal antibodies, at 1.5 µg/ml, were captured onto the modified biosensor surface for 1.5 minutes at 10 µl/min. Recombinant soluble CD40-his was flowed over the biosensor surface at varying concentrations. Antibody and antigen were diluted in 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (HBS-EP). Kinetic and affinity constants were determined using the Biaevaluation software with a 1:1 interaction model/global fit.

As shown in Table 2 below, there is 121-fold difference in the off rate of CHIR-5.9 and CHIR-12.12 resulting in 24-fold higher affinity for CHIR-12.12.

Table 2. Summary of binding properties of CHIR-5.9 and CHIR-12.12 anti-CD40 antibodies.

| Antibody              | K <sub>a</sub> (M <sup>-1</sup> sec <sup>-1</sup> ) | k <sub>d</sub> (sec <sup>-1</sup> ) | K <sub>D</sub> (nM) |
|-----------------------|---|-------------------------------------|---------------------|
| Anti-CD40, CHIR-5.9   | $(12.35 \pm 0.64) \times 10^5$                      | $(15.0 \pm 1.3) \times 10^{-3}$     | $12.15 \pm 0.35$    |
| Anti-CD40, CHIR-12.12 | $(2.41 \pm 0.13) \times 10^5$                       | $(1.24 \pm 0.06) \times 10^{-4}$    | $0.51 \pm 0.02$     |

Example 4: Characterization of Epitope for Monoclonal Antibodies CHIR-12.12 and CHIR-5.9

To determine the location of the epitope on CD40 recognized by monoclonal antibodies CHIR-12.12 and CHIR-5.9, SDS-PAGE and Western blot analysis were performed. Purified CD40 (0.5 µg) was separated on a 4-12% NUPAGE gel under reducing and non-reducing conditions, transferred to PVDF membranes, and probed with



monoclonal antibodies at 10  $\mu\text{g/ml}$  concentration. Blots were probed with alkaline phosphatase conjugated anti-human IgG and developed using the Western Blue<sup>R</sup> stabilized substrate for alkaline phosphatase (Promega).

Results indicate that anti-CD40 monoclonal antibody CHIR-12.12 recognizes epitopes on both the non-reduced and reduced forms of CD40, with the non-reduced form of CD40 exhibiting greater intensity than the reduced form of CD40 (Table 3; blots not shown). The fact that recognition was positive for both forms of CD40 indicates that this antibody interacts with a conformational epitope part of which is a linear sequence. Monoclonal antibody CHIR-5.9 primarily recognizes the non-reduced form of CD40 suggesting that this antibody interacts with a primarily conformational epitope (Table 3; blots not shown).

Table 3. Domain identification.

|                | Domain 1 | Domain 2 | Domain 3 | Domain 4 |
|----------------|----------|----------|----------|----------|
| mAb CHIR-12.12 | -        | +        | -        | -        |
| mAb CHIR-5.9   | -        | +        | -        | -        |
| mAb 15B8       | +        | -        | -        | -        |

To map the antigenic region on CD40, the four extracellular domains of CD40 were cloned and expressed in insect cells as GST fusion proteins. The secretion of the four domains was ensured with a GP67 secretion signal. Insect cell supernatant was analyzed by SDS-PAGE and western blot analysis to identify the domain containing the epitope.

Monoclonal antibody CHIR-12.12 recognizes an epitope on Domain 2 under both reducing and non-reducing conditions (Table 4; blots not shown). In contrast, monoclonal antibody CHIR-5.9 exhibits very weak recognition to Domain 2 (Table 4; blots not shown). Neither of these antibodies recognize Domains 1, 3, or 4 in this analysis.

Table 4. Domain 2 analysis.

|                | Reduced | Non-reduced |
|----------------|---------|-------------|
| mAb CHIR-12.12 | ++      | +++         |
| mAb CHIR-5.9   | +       | +           |

To define more precisely the epitope recognized by mAb CHIR-12.12, peptides were synthesized from the extracellular Domain 2 of CD40, which corresponds to the sequence PCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICT (residues 61-104 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12). SPOTs membranes (Sigma) containing thirty-five 10mer peptides with a 1-amino-acid offset were generated. Western blot analysis with mAb CHIR-12.12 and anti-human IgG beta-galactosidase as secondary antibody was performed. The blot was stripped and reprobed with mAb CHIR-5.9 to determine the region recognized by this antibody

SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-12.12 at 10 µg/ml yielded positive reactions with spots 18 through 22. The sequence region covered by these peptides is shown in Table 5.

Table 5. Results of SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-12.12.

| Spot Number | <u>Sequence Region</u>                            |
|-------------|---|
| 18          | HQHKYCDPNL (residues 78-87 of SEQ ID NO:10 or 12) |
| 19          | QHKYCDPNLG (residues 79-88 of SEQ ID NO:10 or 12) |
| 20          | HKYCDPNLGL (residues 80-89 of SEQ ID NO:10 or 12) |
| 21          | KYCDPNLGLR (residues 81-90 of SEQ ID NO:10 or 12) |
| 22          | YCDPNLGLRV (residues 82-91 of SEQ ID NO:10 or 12) |

These results correspond to a linear epitope of: YCDPNL (residues 82-87 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12). This epitope contains Y82, D84, and N86, which have been predicted to be involved in the CD40-CD40 ligand interaction.

SPOTs analysis with mAb CHIR-5.9 showed a weak recognition of peptides represented by spots 20-22 shown in Table 6, suggesting involvement of the region YCDPNLGL (residues 82-89 of the sequence shown in SEQ ID NO:10 or SEQ ID NO:12) in its binding to CD40. It should be noted that the mAbs CHIR-12.12 and CHIR-5.9 compete with each other for binding to CD40 in BIACORE analysis.

Table 6. Results of SPOTs analysis probing with anti-CD40 monoclonal antibody CHIR-5.9.

| Spot Number | Sequence Region                                   |
|-------------|---|
| 20          | HKYCDPNLGL (residues 80-89 of SEQ ID NO:10 or 12) |
| 21          | KYCDPNLGLR (residues 81-90 of SEQ ID NO:10 or 12) |
| 22          | YCDPNLGLRV (residues 82-91 of SEQ ID NO:10 or 12) |

The linear epitopes identified by the SPOTs analyses are within the CD40 B1 module. The sequence of the CD40 B1 module is:

HKYCDPNLGLRVQQKGTSETDTIC (residues 80-103 of SEQ ID NO:10 or 12).

Within the linear epitope identified for CHIR-12.12 is C83. It is known that this cysteine residue forms a disulphide bond with C103. It is likely that the conformational epitope of the CHIR-12.12 mAb contains this disulfide bond (C83-C103) and/or surrounding amino acids conformationally close to C103.

#### Example 5: CHIR-12.12 Blocks CD40L-Mediated CD40 Survival and Signaling Pathways in Normal Human B Cells

Soluble CD40 ligand (CD40L) activates B cells and induces various aspects of functional responses, including enhancement of survival and proliferation, and activation of NF $\kappa$ B, ERK/MAPK, PI3K/Akt, and p38 signaling pathways. In addition, CD40L-mediated CD40 stimulation provides survival signals by reduction of cleaved PARP and induction of the anti-apoptotic proteins, XIAP and Mcl-1, in normal B cells. CD40L-

mediated CD40 stimulation also recruits TRAF2 and TRAF3 to bind CD40 cytoplasmic domain.

The following studies demonstrate that CHIR-12.12 directly inhibited all of these stimulation effects on normal human B cells. For example, CHIR-12.12 treatment  
5 resulted in increased cleavage of caspase-9, caspase-3, and PARP as well as reduction of XIAP and Mcl-1 in a time- and dose-dependent manner, restoring B cell apoptosis. Treatment with CHIR-12.12 also inhibited phosphorylation of I $\kappa$ B kinase (IKK)  $\alpha$  and  $\beta$  (NF $\kappa$ B pathway), ERK, Akt, and p38 in response to CD40L-mediated CD40 stimulation. Further, it was found that CHIR-12.12 did not trigger these apoptotic effects without  
10 initial CD40L-mediated CD40 stimulation.

*CHIR-12.12 inhibited survival mediated by CD40 ligand by inducing cleavage of PARP.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp.,  
15 Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu$ g/ml) and control IgG were then added. Cells were collected at 0, 20 minutes, 2 hours, 6 hours, 18 hours, and 26 hours. Cleaved caspase-9, cleaved caspase-3, cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, it was observed that CD40L-mediated CD40 stimulation provided  
20 survival signals as it did not result in increases of cleaved caspase-9, cleaved caspase-3, or cleaved PARP over time, indicating that the cells were not undergoing apoptosis. However, treatment with CHIR-12.12 resulted in an increase of these cleavage products, indicating that CHIR-12.12 treatment abrogated the effects of CD40L binding on survival signaling in sCD40L-stimulated normal B cells, restoring B cell apoptosis (data not  
25 shown).

*CHIR-12.12 inhibited expression of "survival" anti-apoptotic proteins.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp.,  
30 Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu$ g/ml) and control IgG were then

added. Cells were collected at 0, 20 minutes, 2 hours, 6 hours, 18 hours, and 26 hours. Mcl-1, XIAP, CD40, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, sCD40L stimulation resulted in sustained expression of Mcl-1 and XIAP over time. However, treatment of the sCD40L-stimulated cells with CHIR 12.12 resulted in a decrease in expression of these proteins overtime (data not shown). Since Mcl-1 and XIAP are "survival" signals capable of blocking the apoptotic pathway, these results demonstrate that CHIR-12.12 treatment removes the blockade against apoptosis in sCD40L-stimulated normal B cells.

10 *CHIR-12.12 treatment inhibited phosphorylation of IKK $\alpha$  (Ser180) and IKK  $\beta$  (Ser 181) in normal B cells.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (10  $\mu$ g/ml) and control IgG were then added. Cells were collected at 0 and 20 minutes. Phosphorylated IKK $\alpha$  (Ser180) and IKK  $\beta$  (Ser 181) and total IKK $\beta$  controls were detected in cell lysates by Western blot.

Briefly, stimulation by sCD40L resulted in phosphorylation of IKK $\alpha$  (Ser180) and IKK  $\beta$  (Ser 181) over time; however, treatment with CHIR-12.12 abrogated this response to sCD40L stimulation in normal B cells (data not shown).

20

*CHIR-12.12 treatment inhibited survival mediated by CD40 ligand in a dose-dependent manner.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors percent purity between 85-95%) were stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (0.01, 0.1, 0.2, 0.5, 1.0  $\mu$ g/ml) and control IgG were then added. Cells were collected at 24 hours. Cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

25

Briefly, CHIR-12.12 treatment resulted in increase of PARP cleavage in sCD40L stimulated cells in a dose-dependent manner and therefore abrogated the survival signaling pathway in sCD40L-stimulated normal B cells (data not shown).

5 *CHIR-12.12 inhibited expression of "survival" anti-apoptotic proteins in a dose-dependent manner.*

In these experiments,  $0.6 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). CHIR-12.12 (0.5, 2, and 10  $\mu\text{g/ml}$ ) and control IgG  
10 were then added. Cells were collected at 22 hours. Mcl-1, XIAP, cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, CHIR-12.12 treatment reduced Mcl-1 and XIAP expression and increased cleaved PARP expression in sCD40L-stimulated cells in a dose-dependent manner, and thus abrogated these blockades to the apoptotic pathway in sCD40L-stimulated normal B  
15 cells (data not shown).

*CHIR-12.12 did not affect expression of anti-apoptotic proteins, cleaved-PARP, and XIAP, in the absence of soluble CD40L signaling.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors  
20 (percent purity between 85-95%) were treated with CHIR-12.12 (10  $\mu\text{g/ml}$ ) and control IgG only (i.e., cells were not pre-stimulated with sCD40L before adding antibody). Cells were collected at 0, 4, 14, and 16 hours. XIAP, cleaved PARP, and  $\beta$ -actin controls were detected in cell lysates by Western blot.

Briefly, the results show that without sCD40L stimulation, the cells expressed  
25 increased concentrations of cleaved PARP, while expression of XIAP remained constant, in both IgG treated control cells and CHIR-12.12 cells (data not shown). These data indicate that CHIR-12.12 does not trigger apoptosis in normal human B cells without CD40L stimulation.

*CHIR-12.12 inhibits phosphorylation of IKK $\alpha$  (Ser180) and IKK $\beta$  (Ser181), Akt, ERK, and p38 in normal B cells.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were serum starved in 1% FBS-containing media and stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). The cultures were treated with CHIR-12.12 (1 and 10  $\mu$ g/ml) and control IgG. Cells were collected at 0 and 20 minutes. Phospho-IKK $\alpha$ , phospho-IKK $\beta$ , total IKK $\beta$ , phospho-ERK, total ERK, phospho-Akt, total Akt, phospho-p38, and total p38 were detected in cell lysates by Western blot.

Briefly, sCD40L stimulation resulted in increases in IKK $\alpha/\beta$  phosphorylation, ERK phosphorylation, Akt phosphorylation, and p38 phosphorylation, thus leading to survival and or proliferation of the cells. Treatment of the cells with CHIR-12.12 abrogated the effects of sCD40L stimulation on these signaling pathways in normal B cells (data not shown).

*CHIR 12.12 inhibits multiple signaling pathways such as PI3K and MEK /ERK in the CD40 signaling cascade.*

In these experiments,  $1.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were serum starved in 1% FBS-containing media and stimulated with 1  $\mu$ g/ml sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK). The cultures were also treated with CHIR-12.12 (1 and 10  $\mu$ g/ml), Wortmanin, (a PI3K/Akt inhibitor; 1 and 10  $\mu$ M), LY 294002 (a PI3K/Akt inhibitor; 10 and 30  $\mu$ M), and PD 98095 (a MEK inhibitor; 10 and 30  $\mu$ g/ml). Cells were collected at 0 and 20 minutes. Phospho-ERK, phospho-Akt, total Akt, phospho-IKK $\alpha/\beta$ , and total were detected in cell lysates by Western blot.

Briefly, the results show that CHIR-12.12 abrogated the phosphorylation of all of these signal transduction molecules, whereas the signal transduction inhibitors showed only specific abrogation of signaling, indicating that CHIR-12.12 likely inhibits upstream of these signal transduction molecules mediated by CD40L stimulation (data not shown).

*CHIR-12.12 inhibits the binding of signaling molecules TRAF2 and TRAF3 to the cytoplasmic domain of CD40 in normal B cells.*

In these experiments,  $4.0 \times 10^6$  normal human B cells from healthy donors (percent purity between 85-95%) were serum starved for four hours in 1% FBS-containing media and stimulated with 1  $\mu\text{g/ml}$  sCD40L (Alexis Corp., Bingham, Nottinghamshire, UK) for 20 minutes. Cells were collected at 0 and 20 minutes. CD40 was immunoprecipitated using polyclonal anti-CD40 (Santa Cruz Biotechnology, CA), and was probed in a Western blot with anti-TRAF2 mAb (Santa Cruz Biotechnology, CA), anti-TRAF3 mAb (Santa Cruz Biotechnology, CA), and anti-CD40 mAb (Santa Cruz Biotechnology, CA).

Briefly, the results show that TRAF2 and TRAF3 co-precipitated with CD40 after sCD40L stimulation. In contrast, treatment with CHIR-12.12 abrogated formation of the CD40-TRAF2/3 signaling complex in sCD40L-stimulated normal B cells. There were no changes in CD40 expression (data not shown).

Without being bound by theory, the results of these experiments, and the results in the examples outlined above, indicate that the CHIR-12.12 antibody is a dual action antagonist anti-CD40 monoclonal antibody having a unique combination of attributes. This fully human monoclonal antibody blocks CD40L-mediated CD40 signaling pathways for survival and proliferation of B cells; this antagonism leads to ultimate cell death. CHIR-12.12 also mediates recognition and binding by effector cells, initiating antibody dependent cellular cytotoxicity (ADCC). Once CHIR-12.12 is bound to effector cells, cytolytic enzymes are released, leading to B-cell apoptosis and lysis. CHIR-12.12 is a more potent anti-tumor antibody than is rituximab when compared in pre-clinical tumor models.

#### Example 6: Liquid Pharmaceutical Formulation for Antagonist Anti-CD40 Antibodies

Protein stability is known to be sensitive to solution pH as surface charge of a protein varies with pH of solution, resulting in stabilization or destabilization. Thus, non-optimal formulation pHs can alter electrostatic interaction, leading to physical degradation of an antagonist anti-CD40 antibody, such as aggregation, precipitation, and the like. Change in pH conditions could also cause breakage of covalent bonds, leading to



chemical degradation, such as fragmentation, deamidation, and the like. This study was therefore designed to identify an optimal solution pH to minimize antagonist anti-CD40 antibody degradation due to aggregation, fragmentation, and deamidation.

The objective of this study was to investigate the effects of solution pH on stability of the antagonist anti-CD40 antibody CHIR-12.12 by both biophysical and biochemical methods in order to select the optimum solution environment for this antibody. Differential Scanning Calorimetry (DSC) results showed that the conformation stability of CHIR-12.12 is optimal in formulations having pH 5.5-6.5. Based on a combination of SDS-PAGE, Size-Exclusion HPLC (SEC-HPLC), and Cation-Exchange HPLC (CEX-HPLC) analysis, the physicochemical stability of CHIR-12.12 is optimal at about pH 5.0-5.5. In view of these results, one recommended liquid pharmaceutical formulation comprising this antibody is a formulation comprising CHIR-12.12 at about 20 mg/ml formulated in about 10 mM sodium succinate, about 150 mM sodium chloride, and having a pH of about pH 5.5.

#### Materials and Methods

The CHIR-12.12 antibody used in the formulation studies is a human monoclonal antibody produced by a CHO cell culture process. This MAb has a molecular weight of 150 kDa and consists of two light chains and two heavy chains linked together by disulfide bands. It is targeted against the CD40 cell surface receptor on CD40-expressing cells, including normal and malignant B cells, for treatment of various cancers and autoimmune/inflammatory diseases.

The anti-CD40 drug substance used for this study was a CHO-derived purified anti-CD40 (CHIR-12.12) bulk lot. The composition of the drug substance was 9.7 mg/ml CHIR-12.12 antibody in 10 mM sodium citrate, 150 mM sodium chloride, at pH 6.5. The control sample in the study was the received drug substance, followed by freezing at  $\leq -60^{\circ}\text{C}$ , thawing at RT and testing along with stability samples at predetermined time points. The stability samples were prepared by dialysis of the drug substance against different pH solutions and the CHIR-12.12 concentration in each sample was determined by UV 280 as presented in Table 7.

Table 7. CHIR-12.12 formulations.

| Buffer Composition                             | pH  | CHIR-12.12 Concentration<br>( mg/ml) |
|--|-----|--------------------------------------|
| 10 mM sodium citrate, 150 mM sodium chloride   | 4.5 | 9.0                                  |
| 10 mM sodium succinate, 150 mM sodium chloride | 5.0 | 9.3                                  |
| 10 mM sodium succinate, 150 mM sodium chloride | 5.5 | 9.2                                  |
| 10 mM sodium citrate, 150 mM sodium chloride   | 6.0 | 9.7                                  |
| 10 mM sodium citrate, 150 mM sodium chloride   | 6.5 | 9.4                                  |
| 10 mM sodium phosphate, 150 mM sodium chloride | 7.0 | 9.4                                  |
| 10 mM sodium phosphate, 150 mM sodium chloride | 7.5 | 9.5                                  |
| 10 mM glycine, 150 mM sodium chloride          | 9.0 | 9.5                                  |

- 5 Physicochemical stability of the CHIR-12.12 antibody in the various formulations was assayed using the following protocols.

*Differential Scanning Calorimetry (DSC)*

- 10 Conformational stability of different formulation samples was monitored using a MicroCal VP-DSC upon heating 15°C to 90°C at 1°C/min.

*SDS-PAGE*

- 15 Fragmentation and aggregation were estimated using 4-20% Tris-Glycine Gel under non-reducing and reducing conditions. Protein was detected by Coomassie blue staining.

*Size Exclusion Chromatograph (SEC-HPLC)*

- 20 Protein fragmentation and aggregation were also measured by a Water Alliance HPLC with a Tosohaas TSK-GEL 3000SWXL column, 100 mM sodium phosphate, pH 7.0 as mobile phase at a flow rate of 0.7 ml/min.

- 25 *Cation Exchange Chromatography (CEX-HPLC)*

- Charge change related degradation was measured using Waters 600s HPLC system with a Dionex Propac WCX-10 column, 50 mM HEPES, pH 7.3 as mobile phase A and 50 mM HEPES containing 500 mM NaCl, pH 7.3 as mobile phase B at a flow rate  
30 of 0.5°C/min.

## Results and Discussion

### *Conformational stability study.*

5 Thermal unfolding of CHIR-12.12 revealed at least two thermal transitions, probably representing unfolding melting of the Fab and the Fc domains, respectively. At higher temperatures, the protein presumably aggregated, resulting in loss of DSC signal. For the formulation screening purpose, the lowest thermal transition temperature was defined as the melting temperature,  $T_m$ , in this study. Figure 6 shows the thermal melting  
10 temperature as a function of formulation pHs. Formulations at pH 5.5-6.5 provided anti-CD40 with higher conformational stability as demonstrated by the higher thermal melting temperatures.

### *SDS-PAGE analysis.*

15 The CHIR-12.12 formulation samples at pH 4.5-9.0 were incubated at 40°C for 2 months and subjected to SDS-PAGE analysis (data not shown). Under non-reducing  
20 conditions, species with molecular weight (MW) of 23 kDa and 27 kDa were observed in formulations above pH 5.5, and species with MW of 51 kDa were observed in all formulations, but appeared less at pH 5.0-5.5. A species with MW of 100 kDa could be seen at pH 7.5 and pH 9.0.

25 Under reducing conditions, CHIR-12.12 was reduced into free heavy chains and light chains with MW of 50 kDa and 24 kDa, respectively. The 100 kDa species seemed not fully reducible and increased with increasing solution pH, suggesting non-disulfide covalent association might occur in the molecules. Since there were other species with unknown identities on SDS-PAGE, stability comparison of each formulation is based on the remaining purity of CHIR-12.12. Formulations at pH 5.0-6.0 provided a more stable  
30 environment to CHIR-12.12. Few aggregates were detected by SDS-PAGE (data not shown).

### *SEC-HPLC analysis .*

SEC-HPLC analysis detected the intact CHIR-12.12 as the main peak species, an aggregation species as a front peak species separate from the main peak species, a large fragment species as a shoulder peak on the back of the main peak species, and small fragment species were detected post-main peak species. After incubation at 5°C and 25°C for 3 months, negligible amounts of protein fragments and aggregates (<1.0% ) were detected in the above formulations and the CHIR-12.12 main peak species remained greater than 99% purity (data not shown). However, protein fragments gradually developed upon storage at 40°C and more fragments formed at pH 4.5 and pH 6.5-9.0, as shown in Table 8. After incubating the CHIR-12.12 formulations at 40°C for 3 months, about 2-3% aggregates were detected in pH 7.5 and pH 9.0, while less than 1% aggregates were detected in other pH formulations (data not shown). The SEC-HPLC results indicate CHIR-12.12 is more stable at about pH 5.0-6.0.

Table 8. SEC-HPLC results of CHIR-12.12 stability samples under real-time and accelerated storage conditions.

| Sample  | Main peak % |             |             |             | Fragments % |             |             |             |
|---------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|         | t=0         | 40°C<br>1 m | 40°C<br>2 m | 40°C<br>3 m | t=0         | 40°C<br>1 m | 40°C<br>2 m | 40°C<br>3 m |
| Control | 99.4        | 99.2        | 99.9        | 99.5        | <1.0        | <1.0        | <1.0        | <1.0        |
| pH 4.5  | 99.4        | 93.2        | 86.0        | 81.3        | <1.0        | 6.4         | 13.2        | 18.1        |
| pH 5.0  | 99.8        | 98.7        | 91.3        | 89.2        | <1.0        | <1.0        | 7.8         | 10.2        |
| pH 5.5  | 99.8        | 98.9        | 91.4        | 90.6        | <1.0        | <1.0        | 7.6         | 8.8         |
| pH 6.0  | 99.6        | 97.7        | 90.4        | 87.3        | <1.0        | 1.9         | 8.2         | 11.7        |
| pH 6.5  | 99.3        | 93.4        | 89.0        | 86.9        | <1.0        | 5.6         | 9.9         | 12.4        |
| pH 7.0  | 99.2        | 93.9        | 87.4        | 85.1        | <1.0        | 5.5         | 11.1        | 13.5        |
| pH 7.5  | 99.1        | 92.8        | 84.4        | 81.9        | <1.0        | 6.4         | 12.9        | 16.2        |
| pH 9.0  | 99.3        | 82.4        | 61.6        | 50.6        | <1.0        | 15.4        | 36.2        | 47.6        |

#### CEX-HPLC analysis.

CEX-HPLC analysis detected the intact CHIR-12.12 as the main peak species, acidic variants eluted earlier than the main peak species, and C-terminal lysine addition variants eluted post-main peak species. Table 9 shows the dependence of the percentages of the remaining main peak CHIR-12.12 species and acidic variants on solution pH. The control sample already contained a high degree of acidic species (~33%), probably due to

early-stage fermentation and purification processes. The susceptibility of CHIR-12.12 to higher pH solutions is evidenced by two facts. First, the initial formulation sample at pH 9.0 (t=0) already generated 12% more acidic species than the control. Second, the percentage of acidic species increased sharply with increasing pH. The charge change-related degradation is likely due to deamidation. The above data indicate that this type of degradation of CHIR-12.12 was minimized at about pH 5.0-5.5.

Table 9. Percentage of peak area by CEX-HPLC for CHIR-12.12 in different pH formulations under real-time and accelerated storage conditions.

| Sample  | Main peak % |           |             |             |             | Acidic variants % |           |             |             |             |
|---------|-------------|-----------|-------------|-------------|-------------|-------------------|-----------|-------------|-------------|-------------|
|         | t=0         | 5°C<br>3m | 25°C<br>3 m | 40°C<br>1 m | 40°C<br>2 m | t=0               | 5°C<br>3m | 25°C<br>3 m | 40°C<br>1 m | 40°C<br>2 m |
| Control | 49.2        | 49.8      | 49.8        | 49.2        | 50.3        | 32.0              | 33.7      | 33.7        | 32.0        | 33.6        |
| pH 4.5  | 48.5        | 49.7      | 43.7        | 39.7        | 30.0        | 32.5              | 32.6      | 38.0        | 44.2        | 56.4        |
| pH 5.0  | 49.6        | 49.8      | 48.3        | 40.6        | 31.4        | 32.7              | 31.8      | 35.0        | 44.3        | 57.1        |
| pH 5.5  | 50.7        | 50.3      | 48.1        | 40.0        | 30.2        | 32.6              | 31.8      | 37.8        | 48.9        | 63.3        |
| pH 6.0  | 50.2        | 49.9      | 47.9        | 37.4        | 23.9        | 33.1              | 33.6      | 38.5        | 54.9        | 72.7        |
| pH 6.5  | 49.4        | 49.9      | 42.3        | 29.7        | 14.6        | 33.3              | 33.6      | 47.7        | 65.2        | 84.6        |
| pH 7.0  | 49.7        | 49.9      | 21.9        | -           | -           | 34.4              | 36.4      | 64.4        | -           | -           |
| pH 7.5  | 49.3        | 48.3      | 12.7        | -           | -           | 35.5              | 40.1      | 79.2        | -           | -           |
| pH 9.0  | 41.3        | 31.8      | -           | -           | -           | 44.7              | 59.9      | -           | -           | -           |

### Conclusion

The pH has a significant effect on conformational and physicochemical stabilities of CHIR-12.12. Charge change-related degradation was determined to be the main degradation pathway for CHIR-12.12, which was minimized at pH 5.0-5.5. Based on overall stability data, one recommended liquid pharmaceutical formulation comprising this antibody is a formulation comprising CHIR-12.12 at about 20 mg/ml formulated in about 10 mM sodium succinate, about 150 mM sodium chloride, and having a pH of about pH 5.5.

Example 7: Clinical Studies with CHIR-5.9 and CHIR-12.12**Clinical Objectives**

The overall objective is to provide an effective therapy for B cell tumors by targeting them with a combination of an antagonist anti-CD40 antibody and an anti-CD20 antibody. These tumors include B-cell lymphoma, Chronic Lymphocytic Lymphoma (CLL), Acute Lymphoblastic Leukemia (ALL), Multiple Myeloma (MM), Waldenstrom's Macroglobulinemia, and Systemic Castleman's Disease. The signal for these diseases is determined in phase II although some measure of activity may be obtained in phase I. The initial antagonist anti-CD40 antibody is the mAb CHIR-12.12, and the initial anti-CD20 antibody is rituximab (Rituxan®). Later investigations study the combined effects of the mAb CHIR-12.12 or CHIR-5.9 with other anti-CD20 antibodies having the binding characteristics of rituximab.

**Phase I**

- Evaluate safety and pharmacokinetics – dose escalation of these two antibodies in subjects with B cell malignancies.
- Choose dose of each antibody based on safety, tolerability, and change in serum markers of respective targets, i.e., CD40 or CD20. In general an MTD for each of these antibodies when used in combination is sought but other indications of efficacy (depletion of CD40+ and/or CD20+ B cells, etc.) may be adequate for dose finding.
- Consideration of more than one combination of doses especially for different indications, e.g., the CLL combination dose may be different than that for NHL. Thus, some dose finding may be necessary in phase II.
- Patients are dosed weekly with real-time pharmacokinetic (Pk) sampling. Initially a 4-week cycle is the maximum dosing allowed. The Pk may be highly variable depending on the disease studied, density of CD40 and/or CD20, etc.
- This trial(s) is open to subjects with B-cell lymphoma, CLL, and potentially other B cell malignancies.
- Decision to discontinue or continue studies is based on safety, dose, and preliminary evidence of anti-tumor activity, particularly synergistic in nature.

- Activity of combination antibody therapy as determined by response rate is determined in Phase II.
- Identify combination dose(s) for Phase II.

## 5 Phase II

Several trials will be initiated in the above-mentioned tumor types with concentration on B-cell lymphoma, CLL, and Multiple Myeloma (MM). Separate trials may be required in low grade and intermediate/high grade NHL as CD40 and/or CD20 may have a different function depending on the grade of lymphoma. More than one  
10 combination of antibody doses and more than one schedule may be tested in a randomized phase II setting.

In each disease, target a population that has failed current standard of care:

- CLL: patients who were resistant to Campath<sup>®</sup> and chemotherapy.
  - 15 • Low grade NHL: Rituxan<sup>®</sup> or CHOP-R failures
  - Intermediate NHL: CHOP-R failures
  - Multiple Myeloma: Chemotherapy failures
- ✓ Decision to discontinue or continue with study is based on proof of  
20 therapeutic concept in Phase II
  - ✓ Determine whether surrogate marker can be used as early indication of clinical efficacy
  - ✓ Identify doses for Phase III

## 25 Phase III

Phase III will depend on where the signal is detected in phase II, and what competing therapies are considered to be the standard. If the signal is in a stage of disease where there is no standard of therapy, then a single arm, well-controlled study could serve as a pivotal trial. If there are competing agents that are considered standard,  
30 then head-to-head studies are conducted.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings.

Therefore, it is to be understood that the inventions are not to be limited to the specific  
5 embodiments disclosed, and that modifications and other embodiments are intended to be included within the scope of the appended claims and embodiments disclosed herein.

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

10 All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.




|  |             |                               |             |
|--|-------------|-------------------------------|-------------|
| Applicant's or agent's<br>file reference | PP22244.002 | International application No. | PCT/US2004/ |
|--|-------------|-------------------------------|-------------|

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(PCT Rule 13bis)

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OR OTHER BIOLOGICAL MATERIAL**

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| Date of deposit<br>17 September 2003  | Accession Number<br>PTA-5543 |
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**ATCC**

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**BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF  
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE****INTERNATIONAL FORM****RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3  
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.****To: (Name and Address of Depositor or Attorney)**Chiron Corporation  
Attn: Karen Van Note  
4560 Horton Street  
Emeryville, CA 94608**Deposited on Behalf of:** Chiron Corporation**Identification Reference by Depositor:**Mouse Hybridoma 131.2F8.5.9: CMCC#12047  
Mouse Hybridoma 153.8E2D10D6.12.12: CMCC#12056**Patent Deposit Designation**PTA-5542  
PTA-5543

The deposits were accompanied by:    a scientific description, a proposed taxonomic description indicated above. The deposits were received September 17, 2003 by this International Depository Authority and have been accepted.

**AT YOUR REQUEST:**   X   We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested September 23, 2003. On that date, the cultures were viable.

**International Depository Authority:** American Type Culture Collection, Manassas, VA 20110-2209 USA.

**Signature of person having authority to represent ATCC:**  
Marie Harris, Patent Specialist, ATCC Patent Depository**Date:** October 2, 2003cc: Lisa Alexander  
Ref: Docket or Case No.: P20107.001

## THAT WHICH IS CLAIMED:

1. A method of treating a human subject for a cancer characterized by neoplastic B cell growth, said method comprising administering to said subject  
5 combination antibody therapy, said therapy comprising administration of an effective amount of an anti-CD40 antibody or antigen-binding fragment thereof in combination with an anti-CD20 antibody or antigen-binding fragment thereof, wherein said anti-CD40 antibody or antigen-binding fragment thereof is free of significant agonist activity when bound to CD40 antigen, said anti-CD40 antibody or antigen-binding fragment thereof  
10 being selected from the group consisting of:
- a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;
  - b) the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
  - c) a monoclonal antibody comprising an amino acid sequence selected from  
15 the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequences shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequences shown in SEQ ID NO:6 and SEQ ID NO:8;
  - d) a monoclonal antibody comprising an amino acid sequence selected from  
20 the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5;
  - e) a monoclonal antibody having an amino acid sequence encoded by a  
25 nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3;
  - f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;
  - 30 g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

h) a monoclonal antibody that binds to an epitope comprising residues 82-89 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

i) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay;

5 j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and

k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j), wherein said fragment retains the capability of specifically binding to said human CD40 antigen.

10

2. The method of claim 1, wherein said combination antibody therapy provides a synergistic therapeutic effect.

3. The method of claim 1, wherein said antigen-binding fragment of said  
15 anti-CD40 antibody or said anti-CD20 antibody is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

4. The method of claim 1, wherein said anti-CD20 antibody is selected from the group consisting of a human anti-CD20 antibody, a murine anti-CD20 antibody, a  
20 chimeric anti-CD20 antibody, and a humanized anti-CD20 antibody.

5. The method of claim 4, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

25 6. The method of claim 5, wherein said anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

7. The method of claim 1, wherein the cancer is selected from the group consisting of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple  
30 myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia,

myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma, follicular small cleaved lymphoma, follicular large cell lymphoma, follicular mixed small cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma, mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.

8. The method of claim 1, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered sequentially.

9. The method of claim 1, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered simultaneously.

10. A method of treating a human subject for a cancer that is characterized by neoplastic B cell growth and which is refractory to treatment with an anti-CD20 antibody or antigen-binding fragment thereof, said method comprising administering to said subject combination antibody therapy, wherein said therapy comprises administration of an effective amount of an anti-CD40 antibody or antigen-binding fragment thereof in combination with said anti-CD20 antibody or antigen-binding fragment thereof, wherein said anti-CD40 antibody or antigen-binding fragment thereof is free of significant agonist activity when bound to CD40 antigen, said anti-CD40 antibody or antigen-binding fragment thereof being selected from the group consisting of:

- a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;
- b) the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;

c) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequences shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequences shown in SEQ ID NO:6 and SEQ ID NO:8;

d) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5;

e) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3;

f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;

g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

h) a monoclonal antibody that binds to an epitope comprising residues 82-89 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

i) a monoclonal antibody that competes with the monoclonal antibody CHIR-5.9 or CHIR-12.12 in a competitive binding assay;

j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and

k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j), wherein said fragment retains the capability of specifically binding to said human CD40 antigen.

11. The method of claim 10, wherein said combination antibody therapy provides a synergistic therapeutic effect.

12. The method of claim 10, wherein said antigen-binding fragment of said anti-CD40 antibody or said anti-CD20 antibody is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

5 13. The method of claim 10, wherein said anti-CD20 antibody is selected from the group consisting of a human anti-CD20 antibody, a murine anti-CD20 antibody, a chimeric anti-CD20 antibody, and a humanized anti-CD20 antibody.

10 14. The method of claim 13, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

15 15. The method of claim 14, wherein said anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

15 16. The method of claim 10, wherein the cancer is selected from the group consisting of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia, myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma, follicular small cleaved lymphoma, follicular large cell lymphoma, follicular mixed small  
20 cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma,  
25 mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.



17. The method of claim 10, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered sequentially.

5 18. The method of claim 10, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered simultaneously.

10 19. A method for inhibiting growth of a tumor comprising neoplastic B cells, comprising contacting said cells with an effective amount of an anti-CD40 antibody or antigen binding fragment thereof in combination with an anti-CD20 antibody or antigen binding fragment thereof, wherein said anti-CD40 antibody or antigen-binding fragment thereof is free of significant agonist activity when bound to CD40 antigen, said anti-CD40 antibody or antigen-antigen binding fragment thereof being selected from the  
15 group consisting of:

a) the monoclonal antibody CHIR-5.9 or CHIR-12.12;

b) the monoclonal antibody produced by the hybridoma cell line 5.9 or  
12.12;

c) a monoclonal antibody comprising an amino acid sequence selected from  
20 the group consisting of the sequence shown in SEQ ID NO:6, the sequence shown in SEQ ID NO:7, the sequence shown in SEQ ID NO:8, both the sequence shown in SEQ ID NO:6 and SEQ ID NO:7, and both the sequence shown in SEQ ID NO:6 and SEQ ID NO:8;

d) a monoclonal antibody comprising an amino acid sequence selected from  
25 the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5;

e) a monoclonal antibody having an amino acid sequence encoded by a  
30 nucleic acid molecule comprising a nucleotide sequence selected from the group

consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;

f) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 5.9 or 12.12;

5 g) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

h) a monoclonal antibody that binds to an epitope comprising residues 82-89 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

i) a monoclonal antibody that competes with the monoclonal antibody  
10 CHIR-5.9 or CHIR-12.12 in a competitive binding assay;

j) the monoclonal antibody of preceding item a) or a monoclonal antibody of any one of preceding items c)-i), wherein said antibody is recombinantly produced; and

k) a monoclonal antibody that is an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-j), wherein said fragment retains  
15 the capability of specifically binding to said human CD40 antigen.

20. The method of claim 19, wherein growth of said tumor is synergistically inhibited.

20 21. The method of claim 19, wherein said antigen-binding fragment of said anti-CD40 antibody or said anti-CD20 antibody is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

22. The method of claim 19, wherein said anti-CD20 antibody is selected from  
25 the group consisting of a human anti-CD20 antibody, a murine anti-CD20 antibody, a chimeric anti-CD20 antibody, and a humanized anti-CD20 antibody.

23. The method of claim 22, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

30

24. The method of claim 23, wherein said anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

25. The method of claim 19, wherein said tumor is associated with a cancer  
5 selected from the group consisting of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia, myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma, follicular small cleaved lymphoma, follicular large cell lymphoma,  
10 follicular mixed small cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma, mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular  
15 lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.

26. The method of claim 25, wherein said cancer is refractory to treatment  
20 with said anti-CD20 antibody or antigen-binding fragment thereof.

27. The method of claim 26, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

28. The method of claim 27, wherein said anti-CD40 antibody is the  
25 monoclonal antibody CHIR-5.9 or CHIR-12.12.

29. A method of treating a human subject for a cancer characterized by neoplastic B cell growth, said method comprising administering to said subject  
30 combination antibody therapy, said therapy comprising administration of an effective amount of an antagonist anti-CD40 antibody or antigen-binding fragment thereof in

combination with an anti-CD20 antibody or antigen-binding fragment thereof, wherein said antagonist anti-CD40 antibody or antigen-binding fragment thereof specifically binds Domain 2 of human CD40 antigen and is free of significant agonist activity when bound to Domain 2 of human CD40 antigen.

5

30. The method of claim 29, wherein said combination antibody therapy provides a synergistic therapeutic effect.

31. The method of claim 29, wherein said antagonist anti-CD40 antibody is a human antibody.

10

32. The method of claim 29, wherein said antagonist anti-CD40 antibody is recombinantly produced.

33. The method of claim 29, wherein said antagonist anti-CD40 antibody has the binding specificity of an antibody selected from the group consisting of the antibody produced by hybridoma cell line 5.9 and the antibody produced by hybridoma cell line 12.12.

15

34. The method of claim 29, wherein said antagonist anti-CD40 antibody is selected from the group consisting of the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5542 and the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5543.

20

35. The method of claim 29, wherein said antagonist anti-CD40 antibody has the binding specificity of monoclonal antibody CHIR-12.12 or CHIR-5.9.

25

36. The method of claim 29, wherein said antagonist anti-CD40 antibody binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12.

30

37. The method of claim 29, wherein said antagonist anti-CD40 antibody or antigen-binding fragment thereof is selected from the group consisting of:

a) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5;

b) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;

c) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 12.12;

d) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;

e) a monoclonal antibody that competes with the monoclonal antibody CHIR-12.12 in a competitive binding assay;

f) a monoclonal antibody of any one of preceding items a)-e), wherein said antibody is recombinantly produced; and

g) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 monoclonal antibody or an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-f), where the fragment retains the capability of specifically binding to said human CD40 antigen.

38. The method of claim 29, wherein said antigen-binding fragment of said antagonist anti-CD40 antibody or said anti-CD20 antibody is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

39. The method of claim 29, wherein said anti-CD20 antibody is selected from the group consisting of a human anti-CD20 antibody, a murine anti-CD20 antibody, a chimeric anti-CD20 antibody, and a humanized anti-CD20 antibody.

40. The method of claim 39, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

41. The method of claim 40, wherein said antagonist anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

42. The method of claim 29, wherein said cancer is selected from the group consisting of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia, myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma, follicular small cleaved lymphoma, follicular large cell lymphoma, follicular mixed small cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma, mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.

43. The method of claim 42, wherein said cancer is refractory to treatment with said anti-CD20 antibody or antigen-binding fragment thereof.

44. The method of claim 43, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

45. The method of claim 44, wherein said antagonist anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

46. The method of claim 29, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered sequentially.

47. The method of claim 29, wherein said anti-CD20 antibody or antigen-binding fragment thereof and said antagonist anti-CD40 antibody or antigen-binding fragment thereof are administered simultaneously.

48. A method for inhibiting growth of a tumor comprising neoplastic B cells, comprising contacting said cells with an effective amount of an antagonist anti-CD40 antibody or antigen binding fragment thereof in combination with an anti-CD20 antibody or antigen binding fragment thereof, wherein said antagonist anti-CD40 antibody or antigen-binding fragment thereof specifically binds Domain 2 of human CD40 antigen and is free of significant agonist activity when bound to Domain 2 of human CD40 antigen.

49. The method of claim 48, wherein growth of said tumor is synergistically inhibited.

50. The method of claim 48, wherein said antagonist anti-CD40 antibody is a human antibody.

51. The method of claim 48, wherein said antagonist anti-CD40 antibody is recombinantly produced.

52. The method of claim 48, wherein said antagonist anti-CD40 antibody has the binding specificity of an antibody selected from the group consisting of the antibody

produced by hybridoma cell line 5.9 and the antibody produced by hybridoma cell line 12.12.

53. The method of claim 48, wherein said antagonist anti-CD40 antibody is selected from the group consisting of the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5542 and the antibody produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5543.

54. The method of claim 48, wherein said antagonist anti-CD40 antibody has the binding specificity of monoclonal antibody CHIR-12.12 or CHIR-5.9.

55. The method of claim 48, wherein said antagonist anti-CD40 antibody binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12.

56. The method of claim 48, wherein said antagonist anti-CD40 antibody or antigen-binding fragment thereof is selected from the group consisting of:

a) a monoclonal antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequence shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequence shown in SEQ ID NO:2 and SEQ ID NO:5;

b) a monoclonal antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequence shown in SEQ ID NO:1 and SEQ ID NO:3;

c) a monoclonal antibody that binds to an epitope capable of binding the monoclonal antibody produced by the hybridoma cell line 12.12;

d) a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:10 or SEQ ID NO:12;



e) a monoclonal antibody that competes with the monoclonal antibody CHIR-12.12 in a competitive binding assay;

f) a monoclonal antibody of any one of preceding items a)-e), wherein said antibody is recombinantly produced; and

5 g) a monoclonal antibody that is an antigen-binding fragment of the CHIR-12.12 monoclonal antibody or an antigen-binding fragment of a monoclonal antibody of any one of preceding items a)-f), where the fragment retains the capability of specifically binding to said human CD40 antigen.

10 57. The method of claim 48, wherein said antigen-binding fragment of said antagonist anti-CD40 antibody or said anti-CD20 antibody is selected from the group consisting of a Fab fragment, an F(ab')<sub>2</sub> fragment, an Fv fragment, and a single-chain Fv fragment.

15 58. The method of claim 48, wherein said anti-CD20 antibody is selected from the group consisting of a human anti-CD20 antibody, a murine anti-CD20 antibody, a chimeric anti-CD20 antibody, and a humanized anti-CD20 antibody.

20 59. The method of claim 58, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

60. The method of claim 59, wherein said anti-CD40 antibody is the monoclonal antibody CHIR-5.9 or CHIR-12.12.

25 61. The method of claim 48, wherein said cancer is selected from the group consisting of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, multiple myeloma, B cell lymphoma, high-grade B cell lymphoma, intermediate-grade B cell lymphoma, low-grade B cell lymphoma, B cell acute lymphoblastic leukemia, myeloblastic leukemia, Hodgkin's disease, plasmacytoma, follicular lymphoma,  
30 follicular small cleaved lymphoma, follicular large cell lymphoma, follicular mixed small cleaved lymphoma, diffuse small cleaved cell lymphoma, diffuse small lymphocytic

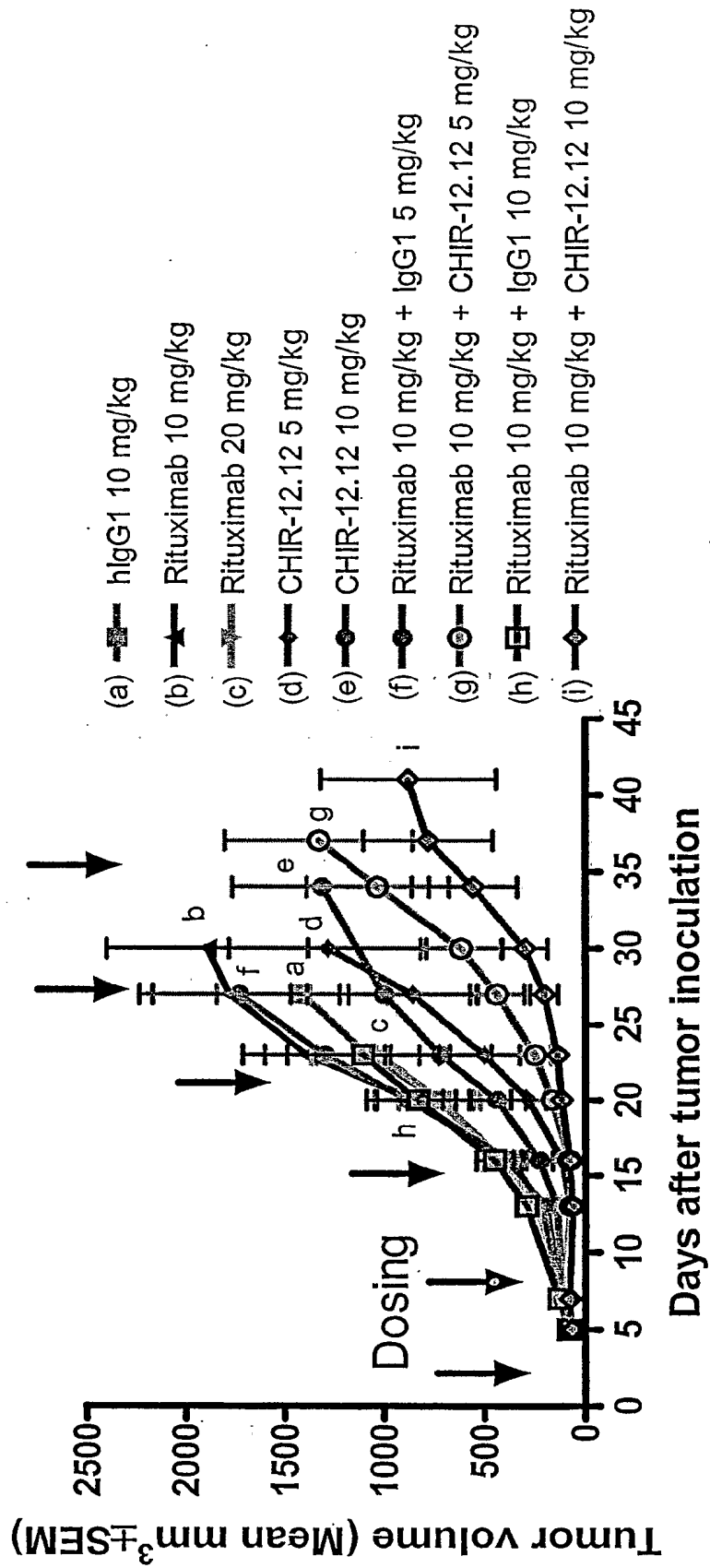
lymphoma, prolymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, hairy cell leukemia, diffuse large cell lymphoma, mediastinal large B cell lymphoma, lymphomatoid granulomatosis, intravascular  
5 lymphomatosis, diffuse mixed cell lymphoma, diffuse large cell lymphoma, immunoblastic lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, and mantle cell lymphoma.

62. The method of claim 61, wherein said cancer is refractory to treatment  
10 with said anti-CD20 antibody or antigen-binding fragment thereof.

63. The method of claim 62, wherein said anti-CD20 antibody is IDEC-C2B8 or an anti-CD20 antibody having the binding characteristics of IDEC-C2B8.

64. The method of claim 63, wherein said anti-CD40 antibody is the  
15 monoclonal antibody CHIR-5.9 or CHIR-12.12.

FIGURE 1



**FIGURE 2A**CHIR 12.12 light chain:

leader:

MALPAQLLGLLMLWVSGSSG

variable:

DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGSNR  
ASGVPPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIR

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ  
DSKDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**FIGURE 2B**CHIR-12.12 heavy chain:

leader:

MEFGLSWVFLVAILRGVQC

variable:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYEESNRY  
HADSVKGRFTISRDN SKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTV  
SS

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS  
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL  
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP  
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTV  
DKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS  
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL  
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP  
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTV  
DKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

## FIGURE 3A

DNA sequence of light chain of CHIR-12.12:

5'atggcgctccctgctcagtcctggggctgctaagtctctgggtctctggatccagtggggatattgtgatgactcagtcctccac  
tctccctgaccgtcaccctggagagccggcctccatctctcaggtccagtcagagcctcgtatagtaatggatacaactat  
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ctgcccgtcacaaagagcttcaacaggggagagtgttag3'

## FIGURE 3B

DNA sequence of heavy chain of CHIR-12.12 (including introns):

5'atggagtttgggctgagctgggtttcctgttctattttaagaggtgtccagtgatcaggtgcagttgggtggagtctgggggag  
gcgtgggtccagcctgggaggtccctgagactctcctgtgcagcctctggattcaccttcagtagctatggcatgcactgggtccg  
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tccctgtctccgggtaaatga3'

**FIGURE 4A**CHIR-5.9 light chain:

leader:

MALLAQLLGLLMLWVPGSSG

variable:

AIVMTQPPLSSPVTLGQPASISCRSSQSLVHSDGNTYLNWLQQRPGQPPRLLIYKFFRR  
LSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQVTQFPHTFGQGTRLEIK

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ  
DSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**FIGURE 4B**CHIR-5.9 heavy chain:

leader:

MGSTAILALLLAVLQGVCA

variable:

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTR  
YSPSFQGGVTTISADKSISTAYLQWSSLKASDTAMYYCARGTAAGR DYYYYYGM D VWGQG  
TTVTVSS

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL  
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP  
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV  
DKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL  
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP  
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV  
DKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

**FIGURE 5A**

Coding sequence for short isoform of human CD40:

```
1 atggttcgtc tgcctctgca gtgcgtctc tggggctgct tgctgaccgc tgtccatcca
61 gaaccaccca ctgcatgcag agaaaaacag tacctaataa acagtcagtg ctgttctttg
121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcaactgaaac ggaatgcctt
181 ccttgcggtg aaagcgaatt cctagacacc tggaacagag agacacactg ccaccagcac
241 aaatactgcg accccaacct agggcttcgg gtccagcaga agggcacctc agaaacagac
301 accatctgca cctgtgaaga aggtggcac tgtacgagtg aggcctgtga gagctgtgtc
361 ctgcaccgct catgctcgcc cggtttggg gtcaagcaga ttgctacagg ggtttctgat
421 accatctgcg agccctgccc agtcggcttc tctccaatg tgtcatctgc ttcgaaaaa
481 tgcaccctt ggacaaggtc ccaggatcg gctgagagcc ctggtggtga tccccatcat
541 ctcgggatc ctgttgcca tcctttggt gctggtcttt atcaaaaagg tggccaagaa
601 gccaaccaat aa
```

**FIGURE 5B**

Encoded short isoform of human CD40:

```
1 mvrplqcvl wgclltavhp epptacrekq ylinsqccsl cpgqklvsd cteftetecl
61 pcgesefldt wnrethchqh kydpnlglr vqkgtsetd tictceegwh ctseacescv
121 lhrscspgfg vkqiatgvsd ticepcpvf fsnvssafek chpwtrspgs aespqgdphh
181 lrdpvchplg aglyqkggqe anq
```

**FIGURE 5C**

Coding sequence for long isoform of human CD40:

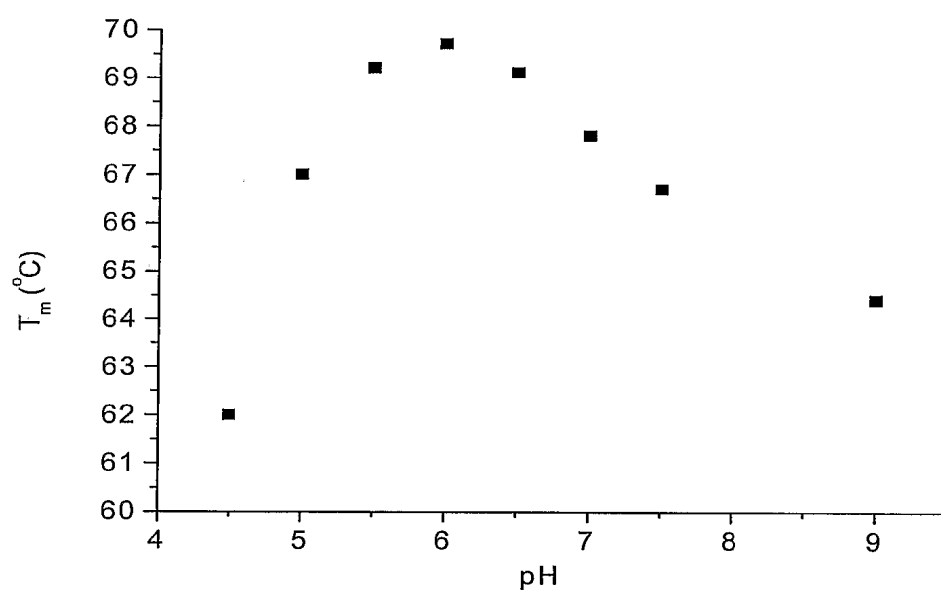
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1 atggttcgtc tgcctctgca gtgcgtctc tggggctgct tgctgaccgc tgtccatcca
61 gaaccaccca ctgcatgcag agaaaaacag tacctaataa acagtcagtg ctgttctttg
121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcaactgaaac ggaatgcctt
181 ctttgcgggtg aaagcgaatt cctagacacc tggaacagag agacacactg ccaccagcac
241 aaatactgcg accccaacct agggcttcgg gtccagcaga agggcacctc agaaacagac
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541 aagactgatg ttgtctgtgg tcccaggat cggctgagag ccttgggtgt gatccccatc
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721 gatcttctg gtcacaacac tgctgtcca gtgcaggaga cttacatgg atgccaaccg
781 gtcaccagg aggatggcaa agagagtcgc atctcagtgc aggagagaca gtga
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**FIGURE 5D**

Encoded long isoform of human CD40:

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121 lhrscspgfg vkqiatgvsd ticepcpvgf fsnvssafek chpwtscetk dlvvqqagtn
181 ktdvvcpqd rlravvipi ifgilfaill vlvfikkvak kptnkaphpk qepqeinfpd
241 dlpgsntaap vqetlhgcqp vtqedgkesr isvqerq
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**FIGURE 6**

## SEQUENCE LISTING

<110> Long, Li  
 Luqman, Mohammad  
 Yabannavar, Asha  
 Zaror, Isabel

<120> Methods of Therapy for B Cell-Related  
 Cancers

<130> PP22244.002 (284270)

<150> 60/613,885  
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 <151> 2004-04-27

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 Gly Ser Ser Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Thr  
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 gtc acc cct gga gag ccg gcc tcc atc tcc tgc agg tcc agt cag agc 144  
 Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser  
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 85 90 95

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Cys Met Gln Ala Arg Gln Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys
115 120 125

gtg gat atc aga cga act gtg gct gca cca tct gtc ttc atc ttc ccg 432
Val Asp Ile Arg Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg 480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat 528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac 576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa 624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag 672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

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&lt;213&gt; Artificial Sequence

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Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45
Leu Leu Tyr Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys
50 55 60
Pro Gly Gln Ser Pro Gln Val Leu Ile Ser Leu Gly Ser Asn Arg Ala
65 70 75 80
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110
Cys Met Gln Ala Arg Gln Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys
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```

```

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                      150                      155                      160
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                      165                      170                      175
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
                      180                      185                      190
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
                      195                      200                      205
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
                      210                      215                      220
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                      230                      235

```

&lt;210&gt; 3

&lt;211&gt; 2016

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Coding sequence for heavy chain of CHIR-12.12  
human anti-CD40 antibody (with introns)

&lt;400&gt; 3

```

atggagtttg ggctgagctg ggttttcctt gttgctattt taagaggtgt ccagtgtcag 60
gtgcagtttg tggagtcttg gggaggcgtg gtccagcctg ggagggtccct gagactctcc 120
tgtgcagcct ctggattcac cttcagtagc tatggcatgc actgggtccg ccagggtcca 180
ggcaaggggc tggagtgggt ggcagttata tcatatgagg aaagtaatag ataccatgca 240
gactccgtga agggccgatt caccatctcc agagacaatt ccaagatcac gctgtatctg 300
caaatgaaca gcctcagaac tgaggacacg gctgtgtatt actgtgctgag agatgggggt 360
atagcagcac ctgggcctga ctactggggc caggggaaccc tggtcaccgt ctctcagca 420
agtaccaagg gccatccgt cttccctctg gcgcccgtca gcaagagcac ctctgggggc 480
acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgttg 540
aactcaggcg ccctgaccag cggcgtgcac accttccccg ctgtcctaca gtccctcagga 600
ctctactccc tcagcagcgt ggtgaccgtg ccctccagca gcttggggcac ccagacctac 660
atctgcaacg tgaatcaca gcccagcaac accaagggtg acaagagagt tggtagagag 720
ccagcacagg gaggagggt gtctgttgga agccaggctc agcgctcctg cctggacgca 780
tcccggctat gcagtcccag tccagggcag caaggcaggc cccgtctgcc tcttcacccg 840
gaggcctctg ccgccccac tcatgctcag ggagagggtc ttctggcttt ttccccaggc 900
tctgggcagg cacaggctag gtgcccctaa ccaggccct gcacacaaag gggcagggtg 960
tgggctcaga cctgccaaga gccatatccg ggaggaccct gccctgacc taagcccacc 1020
ccaaaggcca aactctccac tccctcagct cggacacctt ctctcctccc agattccagt 1080
aactcccaat cttctctctg cagagcccaa atcttgtgac aaaactcaca catgcccacc 1140
gtgcccaggt aagccagccc aggcctcgcc ctccagctca aggcgggaca ggtgcccctag 1200
agtagcctgc atccaggac aggcaccagc cgggtgctga caggtccacc tccatctctt 1260
cctcagcacc tgaactcctg ggggaccgt cagtcttctt cttccccca aaacccaagg 1320
acacctcat gatctcccg accctgagg tcacatgcgt ggtggtggac gtgagccacg 1380
aagaccctga ggtcaagttc aactggtacg tggacggcgt ggaggtgcat aatgccaaga 1440
caaagccgag ggaggagcag tacaacagca cgtaccgtgt ggtcagcgtc ctaccgtcc 1500
tgcaccagga ctggctgaat ggcaaggagt acaagtcaa ggtctccaac aaagccctcc 1560
cagcccccat cgagaaaacc atctccaaag ccaaagggtg gaccctggg gtgaggggc 1620
cacatggaca gaggcggct cggcccaccc tctgccctga gagtaccgc tgtaccaacc 1680
tctgtcccta cagggcagcc ccgagaacca cagggtgtaca ccctgcccc atccggggag 1740
gagatgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta tccagcgac 1800
atcgccgtg agtgggagag caatgggcag ccggagaaca actacaagac caccctccc 1860
gtgctggact ccgacggctc cttcttcttc tatagcaagc tcaccgtgga caagagcagg 1920
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggtcttgca caaccactac 1980
acgcagaaga gcctctccct gtctccgggt aaatga 2016

```

&lt;210&gt; 4

&lt;211&gt; 469

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Heavy chain of CHIR-12.12 human anti-CD40 antibody

&lt;400&gt; 4

```

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly
 1          5          10          15
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20          25          30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35          40          45
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50          55          60
Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala
 65          70          75          80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile
 85          90          95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val
100          105          110
Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr
115          120          125
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
130          135          140
Pro Ser Val Phe Pro Leu Ala Pro Ala Ser Lys Ser Thr Ser Gly Gly
145          150          155          160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165          170          175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180          185          190
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195          200          205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210          215          220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225          230          235          240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245          250          255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260          265          270
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275          280          285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290          295          300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305          310          315          320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325          330          335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340          345          350
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355          360          365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
370          375          380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385          390          395          400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
405          410          415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
420          425          430
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
435          440          445
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
450          455          460
Leu Ser Pro Gly Lys
465

```

&lt;210&gt; 5

&lt;211&gt; 469

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Heavy chain of variant of CHIR-12.12 human  
anti-CD40 antibody

&lt;400&gt; 5

```

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly
 1          5          10          15
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
          20          25          30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
          35          40          45
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
          50          55          60
Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala
          65          70          75          80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile
          85          90          95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val
          100          105          110
Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr
          115          120          125
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
          130          135          140
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
          145          150          155          160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
          165          170          175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
          180          185          190
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
          195          200          205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
          210          215          220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
          225          230          235          240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
          245          250          255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
          260          265          270
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
          275          280          285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
          290          295          300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
          305          310          315          320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
          325          330          335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
          340          345          350
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
          355          360          365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
          370          375          380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
          385          390          395          400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
          405          410          415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
          420          425          430
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
          435          440          445

```

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
 450 455 460  
 Leu Ser Pro Gly Lys  
 465

<210> 6  
 <211> 239  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Light chain of CHIR-5.9 human anti-CD40 antibody

<400> 6  
 Met Ala Leu Leu Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Pro  
 1 5 10 15  
 Gly Ser Ser Gly Ala Ile Val Met Thr Gln Pro Pro Leu Ser Ser Pro  
 20 25 30  
 Val Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser  
 35 40 45  
 Leu Val His Ser Asp Gly Asn Thr Tyr Leu Asn Trp Leu Gln Gln Arg  
 50 55 60  
 Pro Gly Gln Pro Pro Arg Leu Leu Ile Tyr Lys Phe Phe Arg Arg Leu  
 65 70 75 80  
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe  
 85 90 95  
 Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr  
 100 105 110  
 Cys Met Gln Val Thr Gln Phe Pro His Thr Phe Gly Gln Gly Thr Arg  
 115 120 125  
 Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro  
 130 135 140  
 Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu  
 145 150 155 160  
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp  
 165 170 175  
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp  
 180 185 190  
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys  
 195 200 205  
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln  
 210 215 220  
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 225 230 235

<210> 7  
 <211> 474  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Heavy chain of CHIR-5.9 human anti-CD40 antibody

<400> 7  
 Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly  
 1 5 10 15  
 Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
 20 25 30  
 Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe  
 35 40 45  
 Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu  
 50 55 60

```

Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser
65              70              75              80
Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
85              90              95
Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
100            105            110
Tyr Tyr Cys Ala Arg Gly Thr Ala Ala Gly Arg Asp Tyr Tyr Tyr Tyr
115            120            125
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
130            135            140
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ala Ser Lys
145            150            155            160
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
165            170            175
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
180            185            190
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
195            200            205
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
210            215            220
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
225            230            235            240
Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
245            250            255
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
260            265            270
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
275            280            285
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
290            295            300
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
305            310            315            320
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
325            330            335
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
340            345            350
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
355            360            365
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
370            375            380
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
385            390            395            400
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
405            410            415
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
420            425            430
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
435            440            445
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
450            455            460
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465            470

```

```

<210> 8
<211> 474
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Heavy chain of variant of CHIR-5.9 human anti-CD40
antibody

```

```

<400> 8

```



Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly  
 1 5 10 15  
 Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
 20 25 30  
 Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe  
 35 40 45  
 Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu  
 50 55 60  
 Glu Trp Met Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser  
 65 70 75 80  
 Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser  
 85 90 95  
 Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met  
 100 105 110  
 Tyr Tyr Cys Ala Arg Gly Thr Ala Ala Gly Arg Asp Tyr Tyr Tyr Tyr  
 115 120 125  
 Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
 130 135 140  
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys  
 145 150 155 160  
 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
 165 170 175  
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
 180 185 190  
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
 195 200 205  
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
 210 215 220  
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
 225 230 235 240  
 Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys  
 245 250 255  
 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
 260 265 270  
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
 275 280 285  
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp  
 290 295 300  
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu  
 305 310 315 320  
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
 325 330 335  
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
 340 345 350  
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
 355 360 365  
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu  
 370 375 380  
 Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr  
 385 390 395 400  
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn  
 405 410 415  
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe  
 420 425 430  
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn  
 435 440 445  
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
 450 455 460  
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 465 470

&lt;210&gt; 9

&lt;211&gt; 612

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

```

<220>
<221> CDS
<222> (1)...(612)

<221> misc_feature
<222> (0)...(0)
<223> Coding sequence for short isoform of human CD40

<400> 9
atg gtt cgt ctg cct ctg cag tgc gtc ctc tgg ggc tgc ttg ctg acc 48
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
1 5 10 15

gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta 96
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
20 25 30

ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg 144
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
35 40 45

agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa 192
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
50 55 60

agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac 240
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
65 70 75 80

aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc 288
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
85 90 95

tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
100 105 110

agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tcg ccc ggc 384
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
115 120 125

ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag 432
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
130 135 140

ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa 480
Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145 150 155 160

tgt cac cct tgg aca agg tcc cca gga tcg gct gag agc cct ggt ggt 528
Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
165 170 175

gat ccc cat cat ctt cgg gat cct gtt tgc cat cct ctt ggt gct ggt 576
Asp Pro His His Leu Arg Asp Pro Val Cys His Pro Leu Gly Ala Gly
180 185 190

ctt tat caa aaa ggt ggc caa gaa gcc aac caa taa 612
Leu Tyr Gln Lys Gly Gly Gln Glu Ala Asn Gln *
195 200

<210> 10
<211> 203
<212> PRT

```

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

```

Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
 1          5          10          15
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
          20          25          30
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
          35          40          45
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
          50          55          60
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
65          70          75          80
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
          85          90          95
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
          100          105          110
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
          115          120          125
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
          130          135          140
Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145          150          155          160
Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
          165          170          175
Asp Pro His His Leu Arg Asp Pro Val Cys His Pro Leu Gly Ala Gly
          180          185          190
Leu Tyr Gln Lys Gly Gly Gln Glu Ala Asn Gln
          195          200

```

&lt;210&gt; 11

&lt;211&gt; 834

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)...(834)

&lt;221&gt; misc\_feature

&lt;222&gt; (0)...(0)

&lt;223&gt; Coding sequence for long isoform of human CD40

&lt;400&gt; 11

```

atg gtt cgt ctg cct ctg cag tgc gtc ctc tgg ggc tgc ttg ctg acc      48
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
 1          5          10          15

gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta      96
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
          20          25          30

ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg     144
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
          35          40          45

agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa     192
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
          50          55          60

agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac     240
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
          65          70          75          80

aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc     288

```

```

Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
      85                      90                      95

tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
      100                      105                      110

agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tcg ccc ggc 384
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
      115                      120                      125

ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag 432
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
      130                      135                      140

ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa 480
Pro Cys Pro Val Gly Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
      145                      150                      155

tgt cac cct tgg aca agc tgt gag acc aaa gac ctg gtt gtg caa cag 528
Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln
      165                      170                      175

gca ggc aca aac aag act gat gtt gtc tgt ggt ccc cag gat cgg ctg 576
Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu
      180                      185                      190

aga gcc ctg gtg gtg atc ccc atc atc ttc ggg atc ctg ttt gcc atc 624
Arg Ala Leu Val Val Ile Pro Ile Ile Phe Gly Ile Leu Phe Ala Ile
      195                      200                      205

ctc ttg gtg ctg gtc ttt atc aaa aag gtg gcc aag aag cca acc aat 672
Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn
      210                      215                      220

aag gcc ccc cac ccc aag cag gaa ccc cag gag atc aat ttt ccc gac 720
Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp
      225                      230                      235

gat ctt cct ggc tcc aac act gct gct cca gtg cag gag act tta cat 768
Asp Leu Pro Gly Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His
      245                      250                      255

gga tgc caa ccg gtc acc cag gag gat ggc aaa gag agt cgc atc tca 816
Gly Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile Ser
      260                      265                      270

gtg cag gag aga cag tga
Val Gln Glu Arg Gln *
      275

```

&lt;210&gt; 12

&lt;211&gt; 277

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

```

Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
  1          5          10          15
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
      20          25          30
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
      35          40          45
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
      50          55          60

```

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Glu | Phe | Leu | Asp | Thr | Trp | Asn | Arg | Glu | Thr | His | Cys | His | Gln | His |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Lys | Tyr | Cys | Asp | Pro | Asn | Leu | Gly | Leu | Arg | Val | Gln | Gln | Lys | Gly | Thr |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ser | Glu | Thr | Asp | Thr | Ile | Cys | Thr | Cys | Glu | Glu | Gly | Trp | His | Cys | Thr |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Ser | Glu | Ala | Cys | Glu | Ser | Cys | Val | Leu | His | Arg | Ser | Cys | Ser | Pro | Gly |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Phe | Gly | Val | Lys | Gln | Ile | Ala | Thr | Gly | Val | Ser | Asp | Thr | Ile | Cys | Glu |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Pro | Cys | Pro | Val | Gly | Phe | Phe | Ser | Asn | Val | Ser | Ser | Ala | Phe | Glu | Lys |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Cys | His | Pro | Trp | Thr | Ser | Cys | Glu | Thr | Lys | Asp | Leu | Val | Val | Gln | Gln |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Ala | Gly | Thr | Asn | Lys | Thr | Asp | Val | Val | Cys | Gly | Pro | Gln | Asp | Arg | Leu |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Arg | Ala | Leu | Val | Val | Ile | Pro | Ile | Ile | Phe | Gly | Ile | Leu | Phe | Ala | Ile |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Leu | Leu | Val | Leu | Val | Phe | Ile | Lys | Lys | Val | Ala | Lys | Lys | Pro | Thr | Asn |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Lys | Ala | Pro | His | Pro | Lys | Gln | Glu | Pro | Gln | Glu | Ile | Asn | Phe | Pro | Asp |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Asp | Leu | Pro | Gly | Ser | Asn | Thr | Ala | Ala | Pro | Val | Gln | Glu | Thr | Leu | His |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Gly | Cys | Gln | Pro | Val | Thr | Gln | Glu | Asp | Gly | Lys | Glu | Ser | Arg | Ile | Ser |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Val | Gln | Glu | Arg | Gln |     |     |     |     |     |     |     |     |     |     |     |
|     |     | 275 |     |     |     |     |     |     |     |     |     |     |     |     |     |